THE JOURNAL
OF
GENERAL PHYSIOLOGY

EDITORS
JACQUES LOEB W. J. V. OSTERHOUT

VOLUME V, No. 1 SEPTEMBER 20, 1922

PUBLISHED BIMONTHLY
AT MOUNT ROYAL AND GUILFORD AVENUES, BALTIMORE, MD.
BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

COPYRIGHT, 1922, BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Entered as second-class matter November 25, 1918, at the Post Office at Baltimore, Md., under the Act of March 3, 1879.

Made in the United States of America

PUBLICATIONS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D. PEYTON ROUS, M.D.

The Journal of Experimental Medicine is designed to cover the field of experimental medicine. It is issued monthly, two volumes of over 600 pages each appearing in a year. The subscription price per year (two volumes) is $5.00, payable in advance. Single copies cost 75 cents. Remittances should be made by draft or check on New York, or by postal money order, payable to *The Journal of Experimental Medicine*, Mount Royal and Guilford Avenues, Baltimore, Md., or Avenue A and 66th Street, New York, N. Y.

THE JOURNAL OF BIOLOGICAL CHEMISTRY

EDITED BY

STANLEY R. BENEDICT, New York, N. Y. LAFAYETTE B. MENDEL, New Haven, Conn.

H. D. DAKIN, Scarborough, N. Y. DONALD D. VAN SLYKE, New York, N. Y.

The Journal of Biological Chemistry is designed for the prompt publication of original investigations of a chemical nature in the biological sciences. It is issued monthly. Each volume consists of as many numbers as are required to make a total of approximately 800 pages. The subscription price is $5.00 per volume to subscribers in the United States and Canada, payable in advance; $5.25 to those in other countries. The number of volumes issued yearly averages four. The price of single copies varies; it is given on the back cover of each number. Remittances should be made by draft or check on New York, or by postal money order, payable to *The Journal of Biological Chemistry*, Mount Royal and Guilford Aves, Baltimore, Md., or Avenue A and 66th Street, New York, N. Y.

THE JOURNAL OF GENERAL PHYSIOLOGY

EDITED BY

JACQUES LOEB, New York, N. Y. W. J. V. OSTERHOUT, Cambridge, Mass.

The Journal of General Physiology is devoted to the explanation of life phenomena on the basis of the physical and chemical constitution of living matter. Information regarding contributions and subscriptions is given in full on the back cover.

STUDIES FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

The published results of investigations conducted in the Institute, or under its grants, are republished at irregular intervals in volumes designated *Studies from The Rockefeller Institute for Medical Research*. The subscription price is $2.00 per volume, payable in advance. A special rate of $1.00 per volume is made to those who subscribe to *The Journal of Experimental Medicine*, *The Journal of Biological Chemistry*, or *The Journal of General Physiology*. Remittances should be made by draft or check on New York, or by postal money order, payable to The Rockefeller Institute for Medical Research, Avenue A and 66th Street, New York, N. Y.

SEMIANNUAL LIST

The Institute publishes a *Semiannual List* of all papers by members of the staff and those working under grants from the Institute, stating the title and place of publication of these reports. The *Semiannual List* will be sent regularly upon application.

Med.
Water

CONTENTS.

No. 1, September 20, 1922.

Hecht, Selig, and Williams, Robert E. The visibility of monochromatic radiation and the absorption spectrum of visual purple 1
Hitchcock, David I. The colloidal behavior of serum globulin. 35
Parker, G. H. The excretion of carbon dioxide by relaxed and contracted sea anemones 45
Crozier, W. J. Cell penetration by acids. VI. The chloroacetic acids 65
Arrhenius, Olof. Absorption of nutrients and plant growth in relation to hydrogen ion concentration 81
Loeb, Jacques. Cataphoretic charges of collodion particles and anomalous osmosis through collodion membranes free from protein 89
Loeb, Jacques. The influence of electrolytes on the cataphoretic charge of colloidal particles and the stability of their suspensions. I. Experiments with collodion particles 109

No. 2, November 20, 1922.

De Kruif, Paul H., and Northrop, John H. The stability of bacterial suspensions. IV. The combination of antigen and antibody at different hydrogen ion concentrations 127
De Kruif, Paul H., and Northrop, John H. The stability of bacterial suspensions. V. The removal of antibody from sensitized organisms 139
Fenn, Wallace O. The adhesiveness of leucocytes to solid surfaces 143
Fenn, Wallace O. Effect of the hydrogen ion concentration on the phagocytosis and adhesiveness of leucocytes 169
Jacobs, M. H. The influence of ammonium salts on cell reaction. 181
Chambers, Robert. A micro injection study on the permeability of the starfish egg 189

MEEK, CAROLYN S., and LIPMAN, CHARLES B. The relation of the reaction and of salt content of the medium on nitrifying bacteria..... 195
BRODY, SAMUEL, and RAGSDALE, ARTHUR C. The equivalence of age in animals..... 205
HARVEY, E. NEWTON. The permeability of cells for oxygen and its significance for the theory of stimulation..... 215
IRWIN, MARIAN. The permeability of living cells to dyes as affected by hydrogen ion concentration..... 223
OSTERHOUT, W. J. V. Some aspects of selective absorption..... 225
LOEB, JACQUES. Sodium chloride and selective diffusion in living organisms..... 231
LOEB, JACQUES. The influence of salts on the rate of diffusion of acid through collodion membranes..... 255
NORTHROP, JOHN H. The mechanism of the influence of acids and alkalies on the digestion of proteins by pepsin or trypsin. 263

No. 3, JANUARY 20, 1923.

HARVEY, E. NEWTON. Studies on bioluminescence. XV. Electroreduction of oxyluciferin..... 275
WAKSMAN, SELMAN A., and STARKEY, ROBERT L. On the growth and respiration of sulfur-oxidizing bacteria..... 285
FENN, WALLACE O. The phagocytosis of solid particles. IV. Carbon and quartz in solutions of varying acidity..... 311
MOORE, A. R. Muscle tension and reflexes in the earthworm... 327
HUSSEY, RAYMOND G., and NORTHROP, JOHN H. A study of the equilibrium between the so called "antitrypsin" of the blood and trypsin..... 335
NORTHROP, JOHN H., and HUSSEY, RAYMOND G. A method for the quantitative determination of trypsin and pepsin..... 353
HUSSEY, RAYMOND G. Further observations on the influence of salts when injected into the animal body..... 359
BROOKS, S. C. Conductivity as a measure of vitality and death. 365
HITCHCOCK, DAVID I. The ionization of protein chlorides..... 383
LOEB, JACQUES. The influence of electrolytes on the cataphoretic charge of colloidal particles and the stability of their suspensions. II. Experiments with particles of gelatin, casein, and denatured egg albumin..... 395

NORTHROP, JOHN H. The mechanism of the effect of acids and alkalies on the digestion of proteins by pepsin or trypsin. A correction 415

No. 4, MARCH 20, 1923.

COLE, WILLIAM H. Circus movements of *Limulus* and the tropism theory 417
IRWIN, MARIAN. The behavior of chlorides in the cell sap of *Nitella* 427
DU NOÜY, P. LECOMTE. A new viscometer 429
BRODY, SAMUEL, RAGSDALE, ARTHUR C., and TURNER, CHARLES W. The rate of decline of milk secretion with the advance of the period of lactation 441
BRODY, SAMUEL, RAGSDALE, ARTHUR C., and TURNER, CHARLES W. The rate of growth of the dairy cow. II. Growth in weight after the age of two years 445
MOORE, A. R. The reaction of *Nereis virens* to unilateral tension of its musculature 451
MOORE, A. R. Galvanotropism in the earthworm 453
STANTON, RALPH E. The selective absorption of potassium by animal cells. III. The effect of hydrogen ion concentration upon the retention of potassium 461
RAY, GEORGE B. Comparative studies on respiration. XXIV. The effects of chloroform on the respiration of dead and of living tissue 469
LOEB, JACQUES. Stability of suspensions of solid particles of proteins and protective action of colloids 479
LOEB, JACQUES. Membrane potentials and cataphoretic potentials of proteins 505

No. 5, MAY 20, 1923.

COHN, EDWIN J., and HENDRY, JESSIE L. Studies in the physical chemistry of the proteins. II. The relation between the solubility of casein and its capacity to combine with base. The solubility of casein in systems containing the protein and sodium hydroxide 521

HECHT, SELIG. Sensory adaptation and the stationary state.... 555
NORTHROP, JOHN H., and LOEB, JACQUES. The photochemical basis of animal heliotropism 581
CROZIER, W. J., and MOORE, A. R. Homostrophic reflex and stereotropism in diplopods 597
NORTHROP, JOHN H. The stability of bacterial suspensions. VI. The influence of the concentration of the suspension on the concentration of salt required to cause complete agglutination. 605
RAY, GEORGE B. Comparative studies on respiration. XXV. The action of chloroform on the oxidation of some organic acids 611
RAY, GEORGE B. Comparative studies on respiration. XXVI. The production of CO_2 from organic acids in relation to their iodine absorption 623
HOAGLAND, D. R., and DAVIS, A. R. The composition of the cell sap of the plant in relation to the absorption of ions 629
HUSSEY, RAYMOND G., and THOMPSON, WILLIAM R. The effect of radio-active radiations and x-rays on enzymes. I. The effect of radiations from radium emanation on solutions of trypsin. 647
HITCHCOCK, DAVID I. Membrane potentials in the Donnan equilibrium 661
LOEB, JACQUES, and KUNITZ, M. Valency rule and alleged Hofmeister series in the colloidal behavior of proteins. I. The action of acids 665
LOEB, JACQUES, and KUNITZ, M. Valency rule and alleged Hofmeister series in the colloidal behavior of proteins. II. The influence of salts 693

No. 6, JULY 20, 1923.

OSTERHOUT, W. J. V. Exosmosis in relation to injury and permeability 709
IRWIN, MARIAN. The penetration of dyes as influenced by hydrogen ion concentration 727
RAY, GEORGE B. Comparative studies on respiration. XXVII. The mechanism of oxidation in relation to chloroform anesthesia 741

NORTHROP, JOHN H. Note on the purification and precipitation of casein 749
NORTHROP, JOHN H. The inactivation of trypsin. IV. The adsorption of trypsin by charcoal 751
SPONSLER, O. L. Structural units of starch determined by x-ray crystal structure method 757
BRODY, SAMUEL, RAGSDALE, ARTHUR C., and TURNER, CHARLES W. The effect of gestation on the rate of decline of milk secretion with the advance of the period of lactation 777
SHACKELL, L. F. Studies in protoplasm poisoning. I. Phenols. 783
LILLIE, R. S., and CATTELL, WARE. The relation between the electrical conductivity of the external medium and the rate of cell division in sea urchin eggs 807
CHAMBERS, ROBERT, and SANDS, HAROLD C. A dissection of the chromosomes in the pollen mother cells of *Tradescantia virginica* L. Plate 1 815
CHAMBERS, ROBERT. The mechanism of the entrance of sperm into the starfish egg 821
LOEB, JACQUES. Theory of regeneration based on mass action .. 831
LOEB, JACQUES. Theory of geotropism based on mass action ... 853
INDEX TO VOLUME V 865

THE VISIBILITY OF MONOCHROMATIC RADIATION AND THE ABSORPTION SPECTRUM OF VISUAL PURPLE.

By SELIG HECHT* and ROBERT E. WILLIAMS.

(From the Laboratory of Physical Chemistry, Harvard Medical School, Boston, and the George Holt Physics Laboratory, Univeristy of Liverpool, Liverpool.)

(Received for publication, July 1, 1922.)

I.

Reasons for Experiments.

When the visible spectrum is reduced to a very low intensity, and is viewed by a dark adapted eye, it appears colorless. The different portions of the spectrum, however, possess different degrees of brightness, the center being much brighter than the ends. Apparently, a given quantity of light energy will produce a quantitatively different effect depending on its frequency. It seemed to us that an exact knowledge of the relation between frequency and brightness at low intensities of illumination should furnish data bearing on the mechanism of dim vision, a hint of which had already been received in a study of dark adaptation (Hecht, 1919–20). We therefore set ourselves the task of determining accurately this relation between the frequency of light and its ability to produce a colorless sensation in the eye.

Attempts to do precisely this have already been made by several investigators. Perhaps the earliest were Ebert (1888), Langley (1888) and Hillebrand (1890). They were followed soon afterward by Abney and Festing (1893) and von Kries and Nagel (1896), and later by Schaternikoff (1902), Pflüger (1902) and Trendelenburg (1904). It might therefore seem superfluous for us to have undertaken this work, and to add another to the already overwhelming number of papers on vision. Unfortunately, however, the work of the above mentioned investigators is far from adequate, first because of the failure to recog-

* National Research Fellow.

nize certain factors entering into the problem, and second because of the sparse and irregular nature of the data.

It seems quite obvious that in order to make any correct comparisons of the relative effects and stimulating power of the different portions of the spectrum, it is necessary to know their energy content. Yet Langley and Pflüger were the only investigators who took this into consideration, and actually measured the energy distribution of the light which they used. The remaining authors either seemed unaware of this point (Ebert, Hillebrand, Abney and Festing, von Kries and Nagel, and Schaternikoff), or being aware, made no measurements with which to correct their data (Trendelenburg). Schaternikoff, for example, determined the relation between wave-length and brightness for the spectrum of gas light, of sunlight, and of diffuse daylight, and notes without further comment that the maxima are in different positions. Pflüger's results, though corrected for energy distribution, are so irregular that they contribute very little to our knowledge except that light between 495 and 525$\mu\mu$ possesses the maximum capacity for stimulating the dark adapted eye. Langley's results, though much better, are too few to serve our purpose, his measurements having been made with only three or four individuals.

A second factor that enters into the measurements is again ignored by all the investigators except Langley and Pflüger. In making determinations of the relative stimulating power of different lights it is necessary to make comparisons of the intensities which will produce the *same* effect in the eye. In other words, one must find the amount of light at different frequencies which will produce the same brightness in the eye. Both Langley and Pflüger determined the energy at the threshold of visibility. The other investigators kept neither the brightness nor the energy constant, but varied both during the experiments.

In short, it seemed high time to secure results by a method which should not only be free from the errors previously committed, but which should be applicable for use with a large number of individuals so as to yield data sufficient to establish the relationship for the eye in general.

Such data are urgently necessary at this time. Ever since Koenig's (1894) first determination of the absorption spectrum of visual purple, there has been a growing conviction, stimulated by the work of Trendelenburg (1904) and the publicity of Henri and Larguier des Bancels (1911), that visual purple is the receptor substance for dim or twilight vision. The main point in the evidence has been that the absorption spectrum and the velocity of bleaching of visual purple coincide with the stimulating power of spectral radiation at low intensities. The degree of coincidence of these three phenomena is, however, far from convincing. Due to the inadequacy of the data, their rather large deviations may be ascribed to individual variation, and to the necessity for making energy distribution corrections in the available data. These matters, however, lie at the very root of the photochemistry of photoreception, and cannot be dismissed in such an off hand manner. They demand an accurate, quantitative investigation.

This we have endeavored to do. As will be apparent, the effort has been amply repaid by the demonstration of new regularities and suggestive correlations, quite different from what would be expected in terms of the inadequate data heretofore available.

II.

Apparatus, Methods, and Calibrations.

1. The method which we first used consisted in determining the minimum intensity of a series of monochromatic illuminations which could just be perceived by the periphery of the eye. The apparatus was essentially a spectrometer, the light from which, after passing through a pair of Nicol prisms, was spread out on a plate of ground glass. Although we used highly intelligent subjects, our results were irregular. They resembled the data published by Pflüger, and for our purposes were flat failures.

The causes of the failure of such a method are instructive. In the first place, the experiments take a certain length of time, and during that time the eye of the subject varies. We do not speak of dark adaptation—a source of error which we recognized and carefully controlled. We refer more to the normal variations in the visual

mechanisms from minute to minute, and to the effects of the strain involved in looking for these extremely dim lights. These variations involve changes of 100 per cent or more, and with this method there is no way of controlling them or of compensating for them. In the second place, it is extremely difficult to tell when one sees a very dim light and when one does not; and to be able to indicate the exact stage of the intensity when a light first appears or ceases to exist is a taxing task, especially if it has to be done a dozen times with different portions of the spectrum. After our experience, Pflüger's irregular results are not surprising. Our results with this method are easily as erratic as those of Pflüger.

The method that we eventually adopted eliminated both these sources of variation. Instead of working with illuminations which are only just perceptible, we used an illumination which, though well below the color threshold, is still bright enough to be seen easily by the dark adapted eye. We then measured the relative energy at the various frequencies which is necessary to produce this degree of brightness. Our method is therefore a photometric one, in which one of the lights is kept at a constant brightness. In this way there is at once removed the uncertainty and strain of looking for lights that are not there. Moreover, there are eliminated the effects of variation in sensitivity of the visual system during the course of the experiments. The subject is required to match photometrically the constant light which serves as a standard. If the eye varies, it does so both for the standard and for the experimental light at the same time. With this method, the results took on at once a gratifying smoothness and regularity which will be apparent in the data to be presented.

2. The arrangement of the apparatus will be made clear with the help of Fig. 1. A 500 candle power Pointolite lamp in a light-tight box has its incandescent target focussed on the slit of a Hilger monochrometer. This is a spectrometer of the constant deviation type with a slit and thermopile groove in place of the eyepiece. In these experiments both slits of the spectrometer were kept at a width of 0.1 mm. The monochromatic light coming from the spectrometer spreads out gradually into a broad beam. Before it has sensibly diverged, however, it is passed through a pair of nicols, taken with

their mountings from a Hilger spectrophotometer. The beam then spreads out, and 150 cm. from the slit it is made to impinge on the end of a viewing box.

Here it passes through openings in an otherwise opaque cardboard. The shape of this cardboard is shown in Fig. 1. It is the pattern of a Lummer-Brodhun photometer made so as to be 10×10.5 cm. Since the

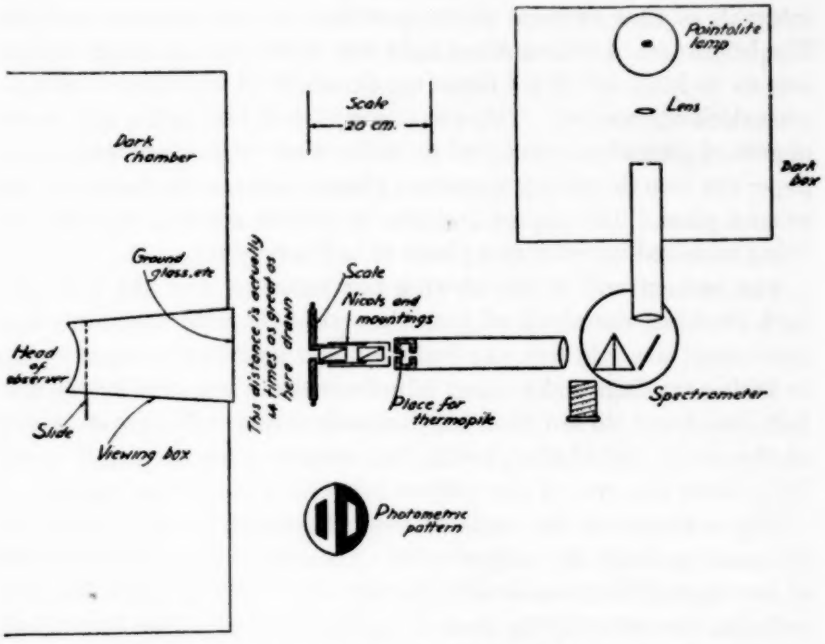

FIG. 1. Diagram of apparatus. The subject sits in the dark chamber curtained off from the rest of the dark room. Note that the front wall of this chamber is actually fourteen times as far from the spectrometer as shown in the drawing.

subject's eyes at the ocular end of the viewing box are 25 cm. from the pattern end, the pattern subtends a visual angle of 22°. The white portion of the pattern represents the parts cut out of the cardboard, through which the monochromatic light passes. In the viewing box and close up against this pattern are two sheets of ground glass which serve the double purpose of diffusing the light and reducing its intensity. The ground glasses are, however, so near the pattern that

though the light which comes through is diffused, it still possesses the shape given to it by the pattern.

This takes care of the variable monochromatic light. The constant comparison light is incorporated into the pattern of the viewing box. It consists of a layer of radium paint which covers the portion of the pattern represented as black in the figure. The layer of radium paint gives a constant and continuous illumination for long intervals of time (several years) provided it is not exposed to light. The brightness of this constant light was so adjusted as to be equivalent to an intensity of 2.7 times the threshold of visibility after complete dark adaptation. This was accomplished first by the two sheets of ground glass already referred to, and second by sheets of translucent paper cut into the proper shape and placed between the paint and the ground glass. The papers and the cardboard are held together by being mounted between two plates of ordinary glass.

The pattern end of the viewing box forms part of the wall of a dark chamber, curtained off from the rest of the dark room, in which the subject sits. In this way flashes of light used by the experimenter in taking readings and making adjustments are not seen by the subject, and hence do not spoil his dark adaptation. Moreover, a slide at the ocular end of the viewing box excludes even the experimental lights from the eyes of the subject between actual observations.

The intensity of the variable monochromatic beam is controlled by a coarse and a fine adjustment. The coarse adjustment consists of two neutral filters made of uniformly fogged photographic film, each reducing the intensity to $\frac{1}{10}$, and together to $\frac{1}{100}$. The fine adjustment between the range of the filters is made with the pair of Nicol prisms. The intensity which the nicols transmit is proportional to $\cos^2\theta$, where θ is the angle between the prisms. At $\theta=0°$ the transmission is the maximum; at $\theta=90°$ the transmission is zero. The function $\cos^2\theta$ varies slowly near 0°, and very rapidly near 90°. In order, therefore, to have a nearly constant rate of adjustment, the nicols were used between 70° and 20°. This is a range of approximately $\frac{1}{10}$, and thus covers the interval between the filters. By using the nicols always, first without any filter, then with Filter 1, and finally with Filters 1 and 2, it is possible to cover by a fine adjustment a range of intensities from 1 to $\frac{1}{1,000}$ of the light transmitted by the nicols alone. This was more than ample for the experiments.

The position of the nicols is indicated in Fig. 1. The filters were placed directly in the path of the monochromatic beam immediately on its emergence from the slit of the spectrometer, in the groove built for the thermopile. The filters were mounted in wooden cases which were so arranged as to fit this groove, thereby insuring a constant and accurate position of the filter.

3. The apparatus as set up requires the calibration of the transmission of the filters, of the settings of the spectrometer, and of the energy distribution in the spectrum. The filters were calibrated photometrically three times, using two sources of light 2 meters apart, and a Lummer-Brodhun photometer. The results were practically the same each time. Filter 1 transmits 0.0977, and Filters 1 and 2 together transmit 0.00954 of the incident light.

The spectrometer, having once been set up, was not moved or readjusted during the experiments here recorded. It was calibrated[1] ten times during the investigation by means of the three hydrogen lines obtained from a hydrogen discharge tube. The results varied comparatively little from time to time, so that we may take their averages as indicating the correct values. In recording the wave-lengths, we use the values obtained graphically from the calibration curve made by plotting the actual values of the hydrogen lines against the drum readings.

The Pointolite lamp which we used runs on 230 volts and 4.5 amperes. We calibrated its energy content in the visible spectrum by means of a Hitchins thermopile and a Broca galvanometer. The resistance of the thermopile is 14.9 ohms, and that of the galvanometer 9.02 ohms. The results are shown in Fig. 2, in which each point is the average of four separate measurements. The energy content of the wave-lengths used in the experiments have been determined by graphic interpolation from this curve.

4. The method of taking observations is very simple, and requires no previous training or experience on the part of the subject. It merely requires him to be intelligent and interested. The subject is brought into the dark room, and 10 minutes are spent in explaining

[1] During the calibration of the spectrometer and of the energy content of the lamp, we had the help of Mr. R. A. Woodeson, to whom we wish to express our thanks.

the object of the experiment, the apparatus, and the procedure. He is then seated in the curtained off, dark chamber in front of the ocular end of the viewing box. Special precautions are taken to make the subject very comfortable, to have his back supported all the time,

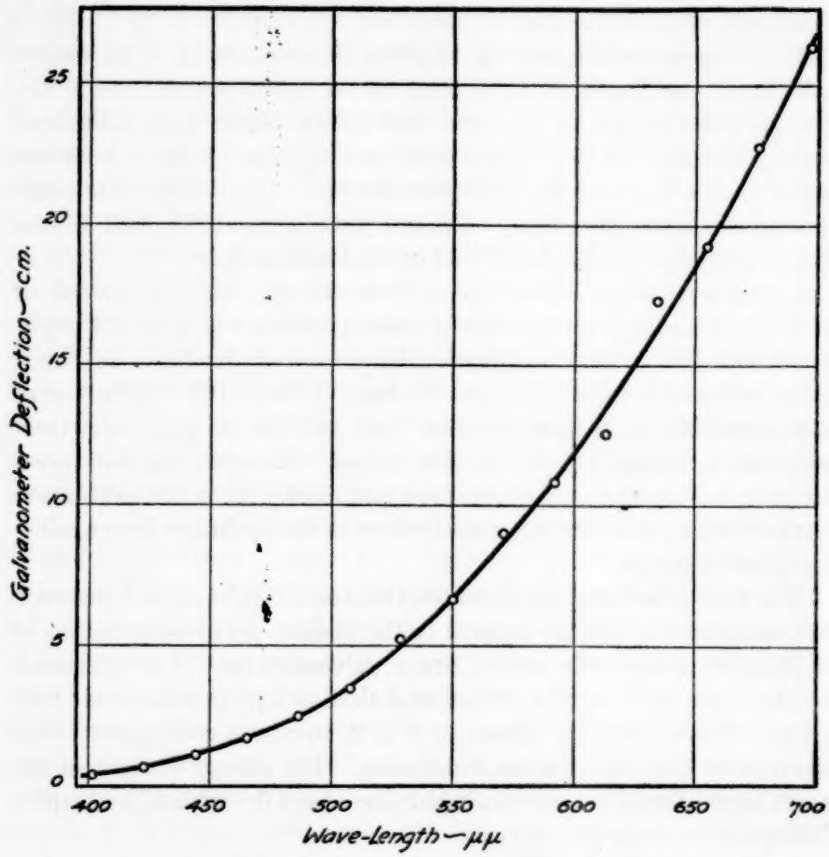

FIG. 2. Energy distribution in spectrum of a 500 candle power Pointolite lamp. Each point is the average of four determinations of the galvanometer deflection obtained from a thermopile placed in the position indicated in Fig. 1.

to have the chair the right height, etc., so that the act of sitting for an hour and a half or more will involve as little physical strain and discomfort as possible. The positions of the chair and the subject

are so adjusted that in order to look into the viewing box he is required to move his head forward only about 5 cm. We mention these details because we are convinced that the personal comfort of the subject has a decided influence on the smoothness and accuracy of the results.

The light from the spectrometer is cut off by placing the nicols at right angles, and the subject is allowed at least half an hour for dark adaptation. During this half hour the subject is kept interested by allowing him to watch the gradual appearance of the pattern formed by the radium paint, indicative of the increase in the sensitivity of his eyes. This pattern usually becomes faintly visible after 10 or 15 minutes. During the next 15 minutes it increases in brightness and clearness, so that the subject becomes familiar with its shape and learns how to find it when looking into the viewing box and pulling out the slide. The appearance of the pattern at this stage is that of an oval field of light with vertical dark bars in it representing the places where there is no radium paint and where the experimental light will come through later. The edges of the pattern are not sharp, because of the ground glass diffusion and the use of rod vision.

The subject is now given a few practise trials in the methods of making observations. The spectrometer is set for 412$\mu\mu$, the nicols being still at right angles. The subject looks in and observes the shadows and the pattern. This takes a few seconds only; the slide is immediately replaced in the viewing box, and the subject remains in complete darkness. The movable nicol is then turned to admit some of the experimental light. The subject again observes the pattern and notices whether the vertical shadows are still as strong as they were before. More illumination is admitted, and the subject again examines the pattern. This is continued until the shadows have completely disappeared, and the field has become uniformly illuminated; the last observations are made slowly and in steps of about 10′ of arc. The final judgment of uniformity is always checked after a rest of a minute or so.

It should be emphasized that the errors and troubles of heterochromic photometry do not exist here, because the field is uniform not only in lack of brightness contrast, but in lack of color as well.

The entire process of making a uniformity judgment is repeated after a few minutes of rest. The same wave-length is used, and the subject is aware that this is a repetition. These preliminary measurements with the same wave-length are continued until two successive determinations agree within a degree of arc. In most cases only two or three such practise readings are necessary, the facility with which the technic is acquired being really surprising. Out of the 48 subjects with whom we made the final measurements, 22 required only 2 determinations to produce the desired duplication; 13 required 3 determinations; 10 required 4 determinations; and 3 required 5 determinations. It is therefore apparent that the method is a simple one, and justifies our assumption that we could apply it to a large number of people, who, though intelligent and to a large extent scientific, yet possess no special training in visual work.

At the end of the practise tests the subject has had at least 45 minutes of dark adaptation, which is more than enough. The measurements are then carried out with lights of chosen wave-length one after the other, allowing about 8 minutes for each determination. After the last portion of the spectrum has been measured, the first measurement is repeated. This observation usually checks well with the first ones; occasionally it is 1° or 2° higher. A complete experiment takes the greater part of a morning or of an afternoon. An example of the type of observations made is given in the record of an experiment in Table I, exactly as it was noted in the record.

III.

Results.

After many preliminary experiments involving modifications of apparatus and technic, we carried out 48 sets of measurements; 43 were with men, and five with women. The average age of the subjects was about 25 years. The subjects were mostly graduate students in the University of Liverpool, but some of them were older people of the teaching staff in Chemistry and Physics. Two were school boys of 15 years, whose results are just as good as those of the others, illustrating the simplicity of the method.

Of the subjects used in these final measurements, three were kind enough to come twice. Fig. 3 gives their data. The points show

the type of result which we obtained, and at the same time give an idea of the degree of reproducibility of the results under the experimental conditions.

In Fig. 3 the ordinates are the logarithms of the relative energy necessary to match the radium paint in brightness. A logarithmic scale is used because a direct plot covering so large a range of values minimizes the finer changes which occur at the lower intensities, and exaggerates the large changes at the higher intensities. Because of

TABLE I.

Data of an Experiment.

W. D., male. Jan. 7, 1922. Lights out at 10:40.

Wave-length.	Time.	Filter.	Nicol reading.
$\mu\mu$			
412	11.20	0	50° 23′
412	11.38	0	50° 43′
455	11.48	1	60° 36′
486	12.05	1 + 2	43° 22′
496	12.15	1 + 2	47° 18′
507	12.22	1 + 2	54° 43′
518	12.30	1 + 2	55° 12′
529	12.40	1 + 2	57° 12′
540	12.50	1 + 2	55° 0′
550	12.58	1 + 2	53° 36′
582	1.12	1 + 2	36° 30′
613	1.16	1	53° 18′
666	1.19	0	49° 50′
412	1.25	0	50° 17′

the construction of the apparatus (decimal filters and nicols) the degree of accuracy is nearly the same at all values of energy content, a fact which can be brought out only by a logarithmic plot.

In Fig. 3 the points for 666 $\mu\mu$ are omitted simply to enable one to get the three sets of results in one figure. The energy required in this region is very high indeed, as will be apparent from Fig. 4.

In order to secure the average results, the following method was used. The data for each subject were computed individually and a graph made of them. From this the minimum point in the curve was found, and an arbitrary value of 10 assigned to it. The experimental results at the other wave-lengths were then computed in terms

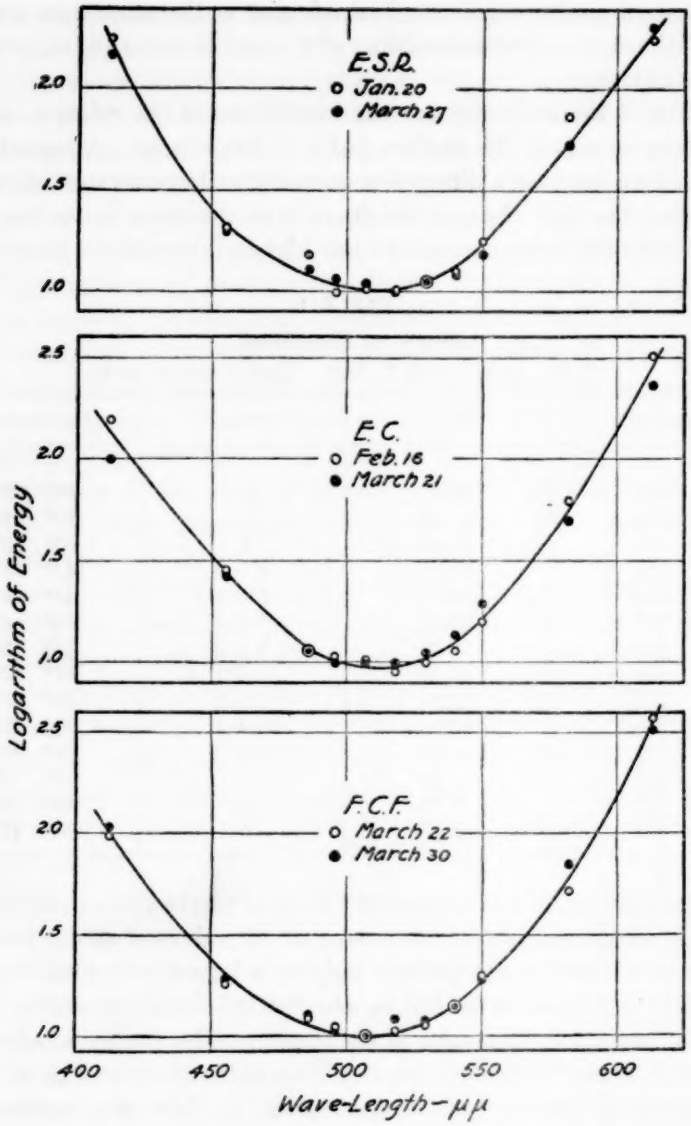

FIG. 3. Results with three subjects who came twice. The points are single readings. The results are reproducible for the same subject at different times, and are practially the same for the three subjects.

of this minimum value of 10. This served to get the data for all the subjects into a similar order of magnitude. The results for each wave-length for the 48 observations were then averaged. These averages are given in Table II, second column. In order to show them graphically, they are plotted logarithmically in Fig. 4.

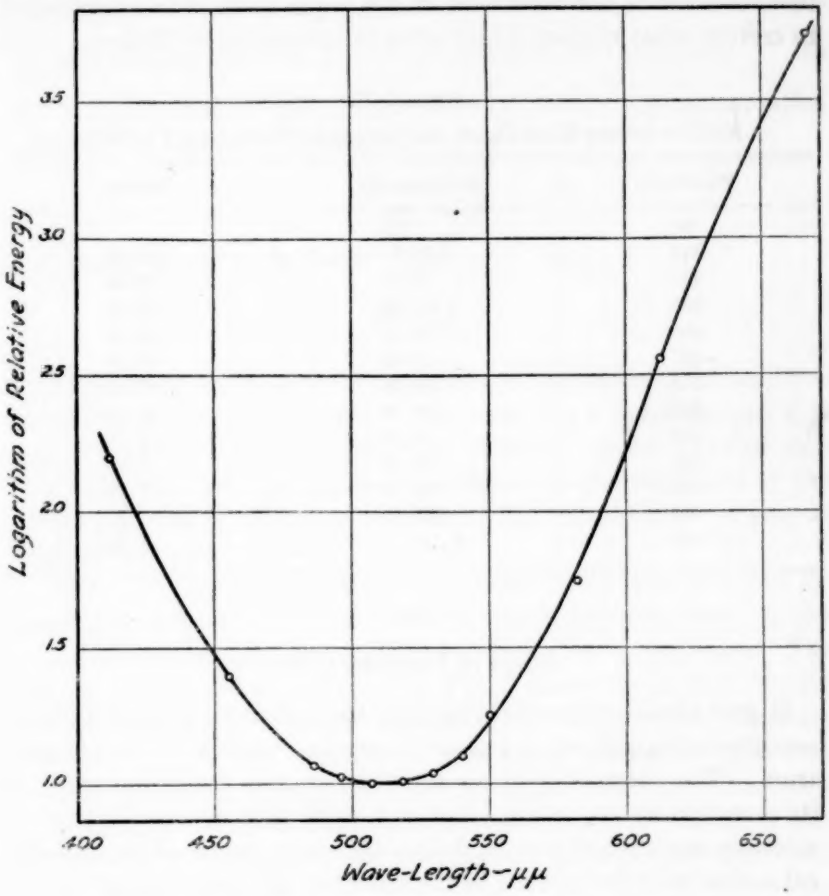

FIG. 4. Relation between energy for achromatic vision and wave-length. Each point is the average of 48 determinations.

It is apparent from Table II and Fig. 4 that the wave-length at which the minimum energy is necessary to produce a given brightness is very near 510 μμ. The energy values for the wave-lengths on both

sides of this point rise quickly, and in the orange and the violet are more than a thousand per cent greater than at the minimum.

Both the position of this minimum and the shape of the curve relating energy for vision with wave-length are of significance for an understanding of the photochemistry of visual reception. We shall therefore devote the remainder of this paper to these two points and to certain other matters which arise in connection with them.

TABLE II.

Relation between Wave-Length and Energy for Vision and Visibility.

Wave-length.	Relative energy.	Visibility.
$\mu\mu$		
412	158.1	6.32
455	25.03	39.95
486	11.99	83.40
496	10.65	93.90
507	10.06	99.35
518	10.28	97.30
529	10.98	91.10
540	12.69	78.78
550	17.99	55.60
582	56.24	17.78
613	367.2	2.72
666	5,525	0.181

IV.

Shape of Visibility Curve.

It goes almost without saying that the rods must possess a photosensitive substance which has an absorption band in the visible spectrum. The effect of light on this substance is the initial event in its reception by the retina. Let us assume that in order to produce a certain sensation of gray in the eye the same amount of photochemical action must be induced in the sensitive substance, irrespective of the wave-length of the light. In terms of Grotthus' law, and its quantitative demonstration by the work of Lasareff (1907), this means that to produce the same visual effect the sensitive substance will have to absorb the same amount of energy regardless of the wave-length of the incident light.

Let us call this amount of energy E. At the position of maximum absorption λ_{max} of the sensitive substance, $\frac{E}{a_{max}}$ units of incident light will be sufficient to yield that amount of absorbed energy, a_{max} being the absorption coefficient at the maximum. At any other wave-length λ the absorption coefficient a_λ will be less than at λ_{max}, but the amount of light necessary so that E units are absorbed will always be $\frac{E}{a_\lambda}$. In short, the amount of light A_λ necessary to produce a given sensation at any wave-length λ will be given by the equation

$$A_\lambda = k\frac{E}{a_\lambda}.$$

By solving this equation for a_λ we get

$$a_\lambda = k\frac{E}{A_\lambda}$$

which gives us the value of the coefficient of absorption of the sensitive substance for any wave-length. The term k is a constant, and E is a constant; therefore the absorption coefficient of the sensitive substance for any wave-length is proportional to the reciprocal of the amount of energy necessary to produce a given sensation of gray at that wave-length.

It should then follow that the reciprocals of our data in Table II, second column, should represent the absorption spectrum of the sensitive substance in the condition in which it is in the retina. The continuous (low intensity) curve in Fig. 5 shows the shape taken by the data when plotted in this manner. The values of the points are taken from the third column of Table II, and are the reciprocals of those in Column 2 arranged so that the maximum of the curve has a value of 100. It is at once clear that the shape of the curve corresponds to the shape of the absorption bands of numerous well known substances.

It would be highly desirable to demonstrate this exact correspondence by applying an equation for the shape of an absorption band to the results in Fig. 5. Unfortunately this cannot be done with any great assurance in the present stage of our knowledge of absorption.

spectra, because there exists no generally applicable equation for the shape of an absorption curve of a complicated substance in solution. In fact there exists no accepted interpretation of the shape of the absorption spectrum even of simple gases.

Several attempts have indeed been made to devise some formula for the shape of visibility curves (as the curves in Fig. 5 have been called) for high intensities (Nutting, 1908) as well as for low intensities

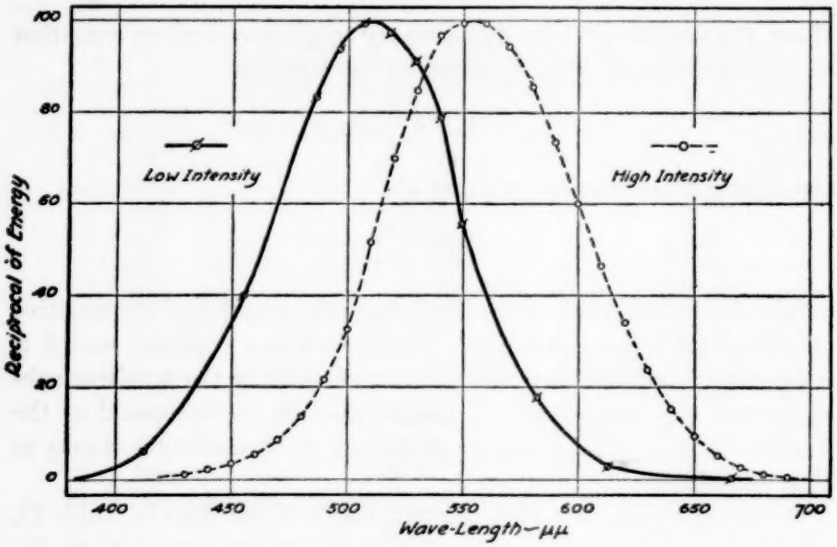

FIG. 5. Visibility curves for achromatic and chromatic vision. The ordinates are proportional to the absorption coefficients of the sensitive substances in the retina. The low intensity curve is therefore the absorption spectrum of the sensitive substance in the rods, while the high intensity curve is the absorption spectrum of the sensitive substance in the cones.

(Goldhammer, 1905; Renqvist, 1920). The best that may be said for these attempts is that they are amusing. They contribute little to the theoretical aspects of the matter, because they are all strictly empirical. Nutting's formula is based on a probability function, whereas Goldhammer's equation has not even that basis. Although nominally derived from Wien's formula for black body radiation, it is really an arbitrary relation between pure numbers possessing no dimensions, and as such it has not a trace of theoretical significance.

Renqvist has tried to apply Planck's resonance theory of light absorption in gases to a calculation of Trendelenburg's data for visibility at low intensities. Aside from the fact that Trendelenburg's data are valueless for this purpose because he took account neither of the adaptation of his eye nor of the energy distribution in the gas flame which he used, the application of Planck's equations in this connection is rather arbitrary and is far from possessing any general significance.

Nutting's attempt to use the probability function is commendable, though he does not seem to recognise the significance of visibility curves in terms of absorption spectra. The symmetrical or nearly symmetrical curves that represent simple absorption spectra resemble strikingly the distribution curves of errors, of populations, etc. which are familiar in the theory of statistics. It may be that the shape of an absorption curve represents the fact that the absorbing substance is composed not of a uniform series of resonators, but of a group of resonators whose free periods may be expressed by the common distribution curves of the statisticians.

It is, however, not in our province to proceed any further along this line of reasoning. For our purposes it will suffice to note the similarity of our curve with curves showing the shape of absorption spectra, leaving aside for the moment the exact theoretical interpretation of such curves. We may then proceed to a comparison of our results with those obtained in the study of the brightness values of spectral light at high intensities in which color enters as a factor.

V.

Chromatic and Achromatic Vision.

In recent years many careful measurements have been made of the relative brightness of different parts of the spectrum at high intensities by methods of heterochromic photometry. The results of all the investigators have been about the same, except, as Hyde, Forsythe and Cady (1918) point out, that the curves obtained with the flicker photometer have in general been somewhat wider than those obtained by methods of direct photometry. The method which we used in our own data on achromatic vision is one of direct photometric

comparison. Without therefore entering into a discussion of the relative merits of the two methods of heterochromic photometry, we have chosen for comparison with our own, those data, which like our own, have been made with a method of direct photometry.

It has never been possible to make this comparison before except in the roughest manner (*cf.* Nutting), because the data for achromatic vision was so inadequate. Now that the necessary data have been made available, we may compare them with the recently reported measurements of Hyde, Forsthye and Cady with 29 subjects. These authors give their data as the reciprocals of the relative energy necessary to produce a certain brightness using different spectral colors. The curve which they present is reproduced in the broken (high intensity) curve of Fig. 5. It is at once clear that the curve also resembles the absorption spectrum of a substance having a band in the visible.

Even a superficial comparison of the two curves in Fig. 5 shows their similarity. To make an accurate demonstration of this resemblance we have proceeded as follows: We have measured the horizontal distances between the two curves in twelve places. The average of these twelve measurements turns out to be very nearly 48 $\mu\mu$. We have then moved the curve of Hyde, Forsythe and Cady 48 $\mu\mu$ to the left, and have measured graphically the ordinates on their curve which correspond to the wave-lengths of the points on our curve. The two sets of points, theirs and ours, are plotted in Fig. 6. Their identity is shown by the fact that the same smooth curve passes through both sets of points. The agreement between the two sets of data is much better than the coincidence between the five or six visibility curves at high intensity which have already appeared from the careful work of different laboratories (*cf.* for example, the set of curves summarized in the paper by Hyde, Forsythe and Cady). It is therefore apparent that the two curves—one at high intensities involving color vision, and the other at low intensities involving only gray vision—are really the same curve in two positions on the spectrum 48 $\mu\mu$ apart.

In order to make the comparison absolutely accurate, the data at high intensities should be corrected for the absorption of the macula lutea. The values for the high intensities are measured with the

fovea, whereas those at low intensities are measured with the periphery. To compare the two results as far as their receptive elements are concerned, the macular absorption should be considered. Unfortunately, however, no decent data are available for this correction. Sachs (1891) measured the absorption of the yellow spot using a double collimator spectrophotometer of the Vierordt type. The results he gives are few and irregular, and it would be an insult to the beautiful

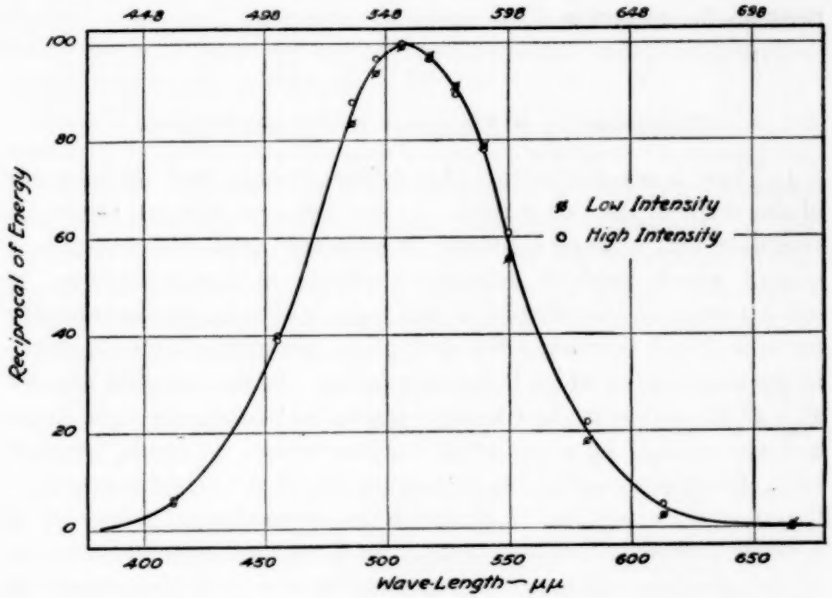

FIG. 6. Identity of high and low intensity curves of Fig. 5. The high intensity curve has been moved 48 μμ to the left, but its scale of abscissæ is given at the top of the figure.

data of Hyde, Forsythe, and Cady to subject them to a correction in terms of the rough and unreliable measurements of Sachs.

Nevertheless we have made this correction for our own interest, simply to see what influence it has on the results. It changes the shape of the curve slightly so as to make it more symmetrical, and it moves the maximum from 554 to 540 μμ. That macular absorption really accounts for this difference in the position of the maximum is demonstrated by the fact that visibility curves for colored lights

made with the fovea and with a region just outside of the macular area differ in the same direction and order of magnitude (Abney and Festing). The corrected curve still coincides with our points, but the difference between the two becomes only 39 instead of 48 $\mu\mu$. Though this reduction is favorable to our interpretation, it possesses no theoretical significance, and leaves the fundamental fact secure that the two brightness curves—one for chromatic vision and the other for achromatic vision—are essentially the same curves in different positions on the spectrum.

VI.

Interpretation of Similarity of Visibility Curves.

1. There is one implication that follows at once from this identity of the shape of the two curves. No one has ever objected to the assumption that a *single* substance is concerned with the reception of stimuli which produce achromatic effects at low intensities. If our reasoning in an early part of this paper is correct, the low intensity curve in Fig. 5 represents the absorption spectrum of this substance in the condition in which it is in the retina. In the visibility of color at high intensities we find a curve which has exactly the same shape and size as that for no color at low intensities. It would therefore seem the most obvious thing to assume that in the brightness perception of color, we are dealing also with the photochemical properties of a *single* photosensitive substance. As far as we ourselves are concerned, we definitely accept this assumption of a single photoreceptive substance as the necessary conclusion from the above comparison.

Precisely what this involves one cannot say. Many questions arise at once. For example, is this single substance the receptive agent for only the brightness values of colors, or is it to be regarded as the receptive material for color vision as a whole? There is no definite answer to this question at present. A certain amount of suggestive data exists for supposing that the brightness function may be distinct from the chromatic function, and the reader is referred to an extended discussion of the possibilities of this separation in a recent review by Troland (1922). The similarity of the brightness curve to the absorption spectrum of a single substance might be taken as additional

evidence for such a separation if one adopts a three or four substance explanation of color vision. To us, however, this is not a necessary step. The field of retinal physiology has hardly been touched from the viewpoint of modern photochemistry; and in view of the meagreness of our quantitative knowledge of the retina and of the photosensory behavior of dyes, it would be premature to decide the matter. All that we wish to conclude at present is that judged solely by the visibility curves, there is as much evidence for assuming only one receptive substance for chromatic vision as there has been for achromatic vision. There the matter must rest until further quantitative information becomes available.

2. There are certain points with regard to this photoreceptive substance, however, a consideration of which will lead to a clarification of our ideas. We can usually reason about the color of a substance from its absorption spectrum. The substance represented by the high intensity curve in Fig. 5 should, like the one represented by the low intensity curve, be purple, but should also be slightly more violet because its maximum is farther toward the red. This substance is to be found in the cones of the retina.

It will be at once objected that no such colored substance, in fact, no colored substance, has ever been demonstrated in the cones. The objection carries its answer with it. There are limits to the visibility of colored solutions. Make them sufficiently dilute, and look at them in thin layers, and they fail to be perceptible. The terminal segments of the cones, where presumably this substance is localized, form an extremely thin layer. If in addition the concentration of the substance is low, the result will be an apparently colorless medium. One who is inclined to be skeptical on this point need only look at a thin layer of a not too dilute solution of hemoglobin, or at a crystal of hemoglobin under the microscope, to realise how limited is our capacity in this respect.

The objection will then be raised that when the solution is so dilute, and the layer so thin, the photochemical characteristics of the substance will have reached their vanishing point. Weigert (1920) has recently given the counter to such an objection by his beautiful work on the photochemical properties of thin layers of AgCl, and of dyes like cyanin. Far from losing their photochemical characteristics,

these dilute, thin layers of material apparently acquire a number of new ones, which, as Weigert has pointed out (1921), will be of significance in any consideration of the mechanism of color vision.

We may therefore accept the proposition that the high intensity curve in Fig. 5 represents the absorption spectrum of a dilute solution of a photosensitive substance in the condition in which it is in the cones. This substance is the receptive material concerned with the production of such quantitative relationships as given by the visibility curve. The recent work of Weigert has given us but a hint of the possibilities for a mechanism to accomplish such effects. But we must not jump, like the many lost souls already on record, into the realms of visual theory.

3. We have then apparently two photosensitive substances in the retina. One is in the rods, and has its maximum of absorption at 511 $\mu\mu$, while the other is in the cones, and has its absorption maximum at 554 $\mu\mu$ (or at 540 $\mu\mu$, if we adopt the correction for the absorption of the macula). There are three logical possibilities for the relation between these two substances. They may be totally different substances; they may be similar substances differing perhaps in the position of the color-bearing groups in the molecule; and they may be identical.

The first possibility of complete diversity of structural relationship is made doubtful by the striking resemblance of the two absorption curves. Judging by the persistence of such substances as hemoglobin and chlorophyll in different groups of organisms, it might be argued that the organism would not produce two widely different substances located in the same sense organ and fulfilling very nearly the same function of brightness evaluation. The argument is admittedly weak; its significance lies in the plausibility of the other possibilities. Between the second and third possibilities there is little choice. The existence of two closely related substances would satisfy the situation, and there is something to be said for such an idea, as we shall presently see. We wish, however, to call attention to the possibility of complete identity.

The main point is the difference of 48 $\mu\mu$ (or of 39 $\mu\mu$ really) between the position of the two maxima. Such differences are, however, well known in spectroscopy, and we may be pardoned for calling attention

to them here. It had been known a long time that the same substance shows different positions for its absorption bands in different solvents; but it was Kundt (1878) who made the first systemmatic investigation of this phenomenon. Kundt wished to correlate the degree of refraction or of dispersion of a solvent with its effect on the position of the absorption bands of a substance dissolved in it. He was only partially successful, and the rule which bears his name, though having a number of exceptions, has been shown to hold true for many substances. In its most general form, Kundt's rule may be stated as follows. If one colorless solvent has a decidedly greater refracting or dispersing capacity than a second, then the absorption bands of a substance dissolved in the first will be nearer the red end of the spectrum than when dissolved in the second (Kayser, 1905).

Kundt's rule has had a particular field of support in the sensitizing properties of certain dyes on the photographic plate. Vogel (1874) found that the maximum of sensitivity of a photographic plate does not correspond with the maximum absorption of the added dye. The maximum of sensitivity is distinctly shifted toward the red. This work was later extended by Eder (1885) who showed that the shift is due to the actual position of the absorption maximum of the dye in the condition in which it is dissolved in the silver bromide grains. Eder obtained shifts toward the red varying from 15 $\mu\mu$ to as high as 65 $\mu\mu$, while shifts to the extent of 31, 47, and 48 $\mu\mu$ were fairly common. Eder has pointed out that this corresponds to Kundt's rule because the density of the AgBr grains is 6.5 as compared with 1.0 for water and 1.3 for dried gelatin. The still more recent work of Sheppard (1909) has amply confirmed the findings of Vogel and of Eder.

The matter may be illustrated beautifully in the case of cyanin. Cyanin has a band in the red—hence its blue color. Dissolved in a film of collodion which is still moist, cyanin is blue. If the film is allowed to dry, thereby increasing the density and refractive index of the collodion, the film becomes colorless, because the absorption band has been shifted into the infra-red (*cf.* Weigert, 1921).

Enough has already been said to show how the same substance could give the two curves in Fig. 5, 48 $\mu\mu$ apart, depending on the refractive index or density of the medium in which it is dissolved.

The Duplicity theory makes a sharp distinction between colored and colorless vision, the one being located in the cones, and the other in the rods. It is conceivable that the terminal segment of the cones is much denser and more refractive than that of the rods, and therefore that the same substance dissolved in the cones would have its absorption band shifted strongly toward the red in comparison to its position in the rods.

This matter, however, is not one for speculation, but can be put to experimental test; we are already engaged in preparing the experiments. There is, nevertheless, a test which may not be without significance, and for which the data are already available. To this we shall now turn.

VII.

Achromatic Brightness and Visual Purple.

1. We have so far not mentioned the relationship of these phenomena to visual purple, because we have wished to consider the matter in some detail and from a point of view somewhat different than the one which has usually been employed (Bayliss, 1918). In 1894 Koenig measured the absorption spectrum of visual purple, and compared it with the brightness value of the spectrum to a color-blind person and to color-sensitive people at low intensities. He concluded that the two sets of curves were coincident, and deduced therefrom the idea that visual purple is the receptor substance for achromatic vision. Trendelenburg in 1904 next measured the rate of bleaching of visual purple in different parts of the spectrum. After measuring in addition his own visibility curve at low intensities, he compared the bleaching rates with his and Schaternikoff's visibility curves, and reached a conclusion similar to that of Koenig. Later (1911) Henri and Larguier des Bancels, without adding any experiments, recomputed the data of Koenig and Trendelenburg and compared them with the results of Schaternikoff and Pflüger on the visibility of the spectrum at low intensities. The conclusion they reached was that in order to produce a visual sensation, the incident light must be of such intensity that the visual purple in the rods will always absorb the same energy regardless of wave-length. This, as the authors point out, is the conclusion of Koenig and of Trendelenburg in a slightly different form.

It will be noticed that these conclusions all depend on the coincidence of the curve of the absorption spectrum of visual purple with that of the curve for the visibility of the spectrum at low intensities. We have already pointed out at the beginning of this paper how unreliable are the data describing the visibility of the spectrum at low intensities. Indeed it was to supply this deficiency that our experiments were made. Now that we have secured these accurate data, it will be interesting to compare them with those of the absorption of visual purple in order to see whether this much discussed coincidence is real or only the product of insufficient data.

Before we do that, however, we wish first to say that, though we accept the conclusions of Koenig, Trendelenburg, and Henri and Larguier des Bancels with regard to the relation of visual purple and achromatic vision, we cannot accept the evidence on which they are based. In fact, we believe that their evidence actually proves something quite different from what they thought. This paradox deserves an explanation.

2. The terminal segments of the rods are, from all observations and descriptions, fairly dense and highly refractive bodies—much more so, for example, than water (von Helmholtz, 1909). It is in these that the visual purple is dissolved in the living retina. If we may argue from Kundt's rule, which we explained in the previous section, the position of the absorption maximum of visual purple in the rods will not be the same as that in solution in water or in dilute bile salts solution. The absorption band of the visual purple in the rods should be shifted toward the red because the medium is much denser and more refractive than bile salts solution. The sensitivity of the rods to spectral light should, therefore, just as the sensitivity of a stained photographic plate, be farther toward the red than would be expected from the position of the absorption spectrum of visual purple as measured in bile salts solution.

If this were found to be true, not only would it strengthen our reasoning in this matter, but it would lend support to the possible explanation which we ventured for the difference in position of the maximum of sensitivity of the rods and the cones. Indeed, it was the idea of this explanation that first led us to examine the data from this critical point of view.

3. The absorption spectrum of visual purple has been measured on three occasions. The first was by Koenig in 1894. Koenig had built a new modification of the Vierordt spectrophotometer for just this work. But before he could acquire any technic with visual purple he came into the possession of a single human retina from a freshly extirpated eye. The temptation was too great, and even though the solution which he prepared was cloudy, he and Köttgen measured its absorption spectrum. The extended and fantastic theories which Koenig developed as a result of his measurements with this single retina are open for him to read who will. It is not for us to dwell too harshly on these lapses. If a datum is worth publishing it is worth interpreting to the limits of its capacity; and the criteria for publication are largely matters of taste, which are not in the realm of discussion. It will suffice to say that, though Koenig's curve for the absorption spectrum is none too regular, it is sufficiently so to show clearly that its maximum is very near 500 $\mu\mu$.

After more practise with the new spectrophotometer and with the technic of preparing visual purple solutions, Köttgen and Abelsdorff (1896) took up the study of the absorption spectrum of visual purple in earnest. They made a large number of measurements with visual purple from a variety of animals, and their results are indicative of careful work. They found that visual purple prepared from the monkey, the cat, the rabbit, and the frog, gives the same absorption spectrum. We have studied their individual measurements in great detail and in every instance for every preparation the maximum of absorption is the same; that is, very near 500 $\mu\mu$. The individual readings between 620 and 460 $\mu\mu$ agree very well with one another, and may be accepted with confidence, short of course, of an error in the calibration of the prism. However, the last two readings in the red (660 and 640 $\mu\mu$) and in the violet (420 and 440 $\mu\mu$) are subject apparently to a large experimental error, because the individual values vary as much as 100 per cent from one another, besides occasionally yielding the theoretically impossible negative value for the absorption coefficient. We have therefore not included these four points in a consideration of their data.

The third determination of the absorption of visual purple was made by Trendelenburg (1904). He made no systemmatic measure-

ments, but on six occasions during his work on the bleaching of visual purple, he measured the absorption spectrum over short intervals now here, now there. The concentrations were never the same, and after making proper corrections, he averaged these irregular results. He then plotted them in comparison with those obtained by Köttgen and Abelsdorff. Though it is obvious that his curve lies consistently 5 $\mu\mu$ farther toward the red, he makes no comment on the comparison. We fail to understand this difference, and must attribute it to the irregularity of the measurements; but we are surprised that the disagreement was allowed to pass without comment.

Of the three sets of measurements, those of Köttgen and Abelsdorff are therefore the only ones that can be considered as fulfilling the criteria of accuracy. Although all three sets of measurements show, without question, that the maximum of the spectrum visibility is distinctly farther toward the red than the absorption maximum of visual purple in bile salts solution, we can make reliable comparisons only with those of Köttgen and Abelsdorff. These are presented in Fig. 7. The solid curve with the small circles is the visibility curve at low intensity taken directly from Fig. 5., and according to our reasoning represents the absorption spectrum of visual purple in the condition in which it is in the terminal segments of the rods. The other points and the broken line are the data of Köttgen and Abelsdorff for the monkey and the rabbit, their original values having been recomputed so as to give the maximum a value of 100. Far from the two curves being coincident, it is apparent that the absorption spectrum of visual purple in the eye is definitely and consistently farther toward the red than in water solution. The two curves are, however, identical, and represent no doubt the same substance in two different media obeying Kundt's rule.

4. This having been established, it will be instructive to examine more closely the curves presented by the various authors in support of the coincidence of the two sets of observations. Koenig's figure shows beyond question that not only the maximum but almost the entire curves for the brightness perception of the spectrum of a color-blind person and of color-sensitive people at low intensities are distinctly farther toward the red than the maximum and curve for the absorption of visual purple. We have carefully replotted on a larger

scale the data given by Koenig, and find that the average difference between the two sets of data is about 5$\mu\mu$.

We have already pointed out the discrepancy of Trendelenburg's absorption data. But even here, if one examines the curves in Fig. 7 of his paper, one sees that the *Dämmerungswerte* are distinctly shifted an average of 8 $\mu\mu$ farther toward the red than the calculated absorp-

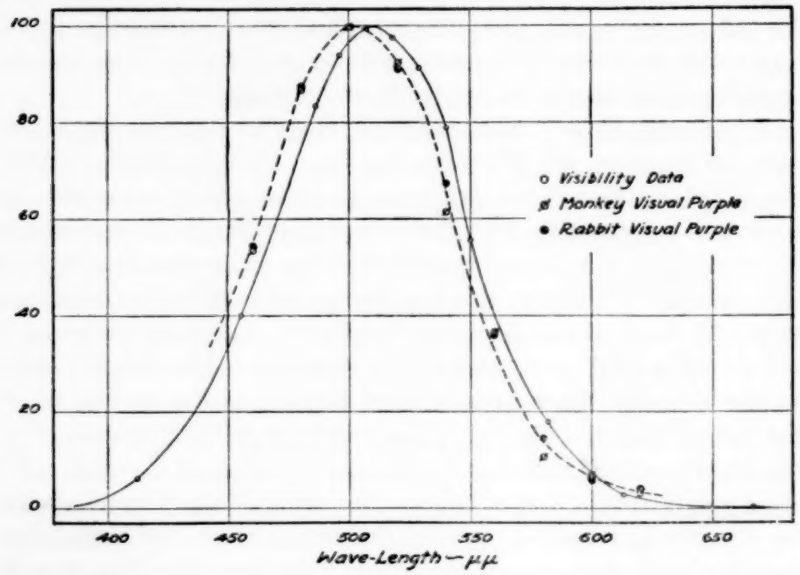

FIG. 7. Relation between absorption spectrum of visual purple in bile salts solution and absorption spectrum of sensitive substance in the rods as given by the visibility curve of Fig. 5. Though the two curves are identical, the visibility curve is shifted 7 or 8 $\mu\mu$ toward the red, as would be expected in terms of Kundt's rule.

tion of visual purple as determined by Trendelenburg. The main work of this author, however, is the bleaching effect of different wavelengths, which is probably quite accurate. If we compare the curve for the bleaching values with that for the dim values (*Dämmerungswerte*) of the same spectrum, we find here again that not only the maximum but the entire curve is displaced toward the red, an average distance of about 5 or 6 $\mu\mu$.

Finally, in the paper by Henri and Larguier des Bancels, if we compare the curve representing the energy necessary to produce the same absorption in visual purple with the curve for the energy necessary to see the spectrum at low intensities, we find exactly the same shift of the latter toward the red. The curves are quite irregular, and it is hard to estimate the exact extent of the shift, but it varies between 5 and 20 $\mu\mu$ in different parts. Any one who cares may see this for himself in the reproduction of Henri and Larguier des Bancels' figure in Bayliss' book on General Physiology.

In all the cases then, the shift, whether large or small, is consistently present, and is always in the same direction, toward the red. Therefore, as we pointed out, though we believe the conclusion expressed by these authors, we find that the evidence they present fails utterly to prove it. More than that, their data actually prove something else, which is quite as significant as what they were supposed to prove. This is that the absorption spectrum of visual purple in the rods, following Kundt's rule, is definitely shifted toward the red for a distance of 7 or 8 $\mu\mu$. It is to this shifted absorption spectrum that, we believe, the proportionality between visibility and absorption must be referred.

It is hardly necessary to indicate that this point too may be tested experimentally; but we do so in order to announce that we are preparing to perform the necessary experiments.

5. There is just one more point we wish to make about the absorption spectrum of visual purple and its relation to the visibility of radiation. We suggested the possibility that the two visibility curves of Fig. 5 are manifestations of the same sensitive substance, and that the difference in the position of the two curves is explainable in terms of Kundt's rule. This would assume that the visual purple which we can extract from the eye and whose maximum of absorption in bile salts solution is at 503 $\mu\mu$ is present in solution in the rods where its absorption maximum is at 511 $\mu\mu$, and in very dilute solution in the cones where its maximum is at 554 $\mu\mu$ (or more probably, as corrected for macular transmission, at 540 $\mu\mu$).

The shift between the rods and the cones is a pretty big one. Not that such shifts are uncommon in the sensitization of a photographic plate, as Eder's figures amply testify; but that such a shift has to be

produced by a pretty big increase in density and refractive index. It may be that the terminal segments of the cones actually possess this greatly increased density or refractivity; that is something that will have to be settled experimentally. There is however another consideration.

We pointed out that one possibility in this relationship is that there may be two closely related forms of the sensitive substance differing slightly in some detail of molecular arrangement, which would account equally well for the shift in the absorption spectrum. There do exist what appear to be two such forms of visual purple. Köttgen and Abelsdorff, in their study of visual purple, found a sharp distinction between the substance obtained from fishes and from all other vertebrates. The maximum for the visual purple of all other vertebrates is, as we have given it above, 503 $\mu\mu$. That from fishes is definitely more violet, and its absorption maximum is at 540 $\mu\mu$. This difference is too consistent for it to be anything but a real one.

This is all that is known. Whether the fish form of visual purple is the one that is present in the cones, or what this fish modification of visual purple is, are questions that cannot even be speculated on at the present time. We even refrain from the tempting, but probably quite futile, discussion of the evolutionary aspects of the situation. It is clear, however, that the field is ready for experimentation, and we hope that we may be fortunate enough to find some solution for these stimulating and provoking questions which strike at the basis of the mechanism of vision.

SUMMARY.

1. After a consideration of the existing data and of the sources of error involved, an arrangement of apparatus, free from these errors, is described for measuring the relative energy necessary in different portions of the spectrum in order to produce a colorless sensation in the eye.

2. Following certain reasoning, it is shown that the reciprocal of this relative energy at any wave-length is proportional to the absorption coefficient of a sensitive substance in the eye. The absorption spectrum of this substance is then mapped out.

3. The curve representing the visibility of the spectrum at very low intensities has exactly the same shape as that for the visibility at high intensities involving color vision. The only difference between them is their position in the spectrum, that at high intensities being 48 $\mu\mu$ farther toward the red.

4. The possibility is considered that the sensitive substances responsible for the two visibility curves are identical, and reasons are developed for the failure to demonstrate optically the presence of a colored substance in the cones. The shift of the high intensity visibility curve toward the red is explained in terms of Kundt's rule for the progressive shift of the absorption maximum of a substance in solvents of increasing refractive index and density.

5. Assuming Kundt's rule, it is deduced that the absorption spectrum of visual purple as measured directly in water solution should *not* coincide with its position in the rods, because of the greater density and refractive index of the rods. It is then shown that, measured by the position of the visibility curve at low intensities, this shift toward the red actually occurs, and is about 7 or 8 $\mu\mu$ in extent. Examination of the older data consistently confirms this difference of position between the curves representing visibility at low intensities and those representing the absorption spectrum of visual purple in water solution.

6. It is therefore held as a possible hypothesis, capable of direct, experimental verification, that the same substance—visual purple—whose absorption maximum in water solution is at 503 $\mu\mu$, is dissolved in the rods where its absorption maximum is at 511 $\mu\mu$, and in the cones where its maximum is at 554 $\mu\mu$ (or at 540 $\mu\mu$, if macular absorption is taken into account, as indeed it must be).

The experiments for this paper were performed in the George Holt Physics Laboratory of the University of Liverpool. We take this opportunity of thanking Professor L. R. Wilberforce for his kindness in placing the facilities of the laboratory at our disposal. It is a pleasure also to record our thanks to Professor E. C. C. Baly for the use of apparatus from the Laboratory of Inorganic Chemistry, and above all, for the generous hospitality of his laboratory.

BIBLIOGRAPHY.

Abney, W. deW., and Festing, E. R., Colour photometry. III, *Phil. Tr. Roy. Soc. London*, 1893, clxxxiii, A, 531.

Bayliss, W. M., Principles of general physiology, 2nd edition, London, 1918.

Ebert, H., Ueber den Einfluss der Schwellenwerthe der Lichtempfindung auf den Charakter der Spectra, *Ann. Physik. u. Chem.*, 1888, xxxiii, 136.

Eder, J. M., Ueber die Wirkung verschiedener Farbstoffe auf das Verhalten des Bromsilbers gegen das Sonnenspectrum, (1885) reprinted in *Beitr. Photochem. u. Spectralanal., 3 te Abt.*, 1904, 26.

Goldhammer, D. A., Die Farbenempfindlichkeit des Auges und die photometrische Helligkeit der leuchtender Körper, *Ann. Physik.*, 1905, xvi, 621.

Hecht, S., The dark adaptation of the human eye, *J. Gen. Physiol.*, 1919–20, ii, 499.

von Helmholtz, H., Handbuch der Physiologischen Optik, Hamburg and Leipsic, 3rd edition, 1909, i.

Henri, V. and Larguier des Bancels, J., Photochimie de la rétine, *J. physiol. et path. gén.*, 1911, xiii, 841.

Hillebrand, F., Über die specifische Helligkeit der Farben, *Sitzungsber. K. Akad. Wiss., Math.-Naturw. Cl., 3te Abt.*, 1890, xcviii, 70.

Hyde, E. P., Forsythe, W. E., and Cady, F. E., The visibility of radiation, *Astrophys. J.*, 1918, xlviii, 65.

Kayser, H., Handbuch der Spectroscopie, Leipsic, 1905, iii.

Koenig, A., Ueber den menschlichen Sehpurpur und seine Bedeuting für das Sehen, *Sitzungsber. Akad. Wiss., Berlin*, 1894, 577; reprinted in *Ges. Abhandl.*, Leipsic, 1903.

Köttgen, E., and Abelsdorff, G., Absorption und Zersetzung des Sehpurpurs bei den Wirbeltieren, *Z. Psychol. u. Physiol. Sinnesorg.*, 1896, xii, 161.

Kundt, A., Ueber den Einfluss des Lösungsmittels auf die Absorptionsspectra gelöster absorbirender Medien, *Ann. Physik. u. Chem.*, 1878, iv, 34.

von Kries, J., and Nagel, W., Über den Einfluss von Lichtstärke und Adaptation auf das Sehen des Dichromaten (Grünblinden), *Z. Psychol. u. Physiol. Sinnesorg.*, 1896, xii, 1.

Langley, S. P., Energy and vision, *Am. J. Sc.*, 1888, xxxvi, 359.

Lasareff, P., Über das Ausbleichen von Farbstoffen im sichtbaren Spektrum, *Ann. Physik.*, 1907, xxiv, 661.

Nutting, P. G., The luminous equivalent of radiation, *Bull. U. S. Bureau of Standards, No. 103*, 1908, 261.

Pflüger, A., Ueber die Farbenempfindlichkeit des Auges, *Ann. Physik.*, 1902, ix, 185.

Renqvist, Y., Ein Versuch, die Plancksche Resonatorentheorie der Lichtabsorption auf die Absorption des Sehpurpurs anzuwenden, *Skand. Arch. Physiol.*, 1920, xl, 226.

Sachs, M., Ueber die specifische Lichtabsorption des gelben Fleckes der Netzhaut, *Arch. ges. Physiol.*, 1891, l, 574.

Schaternikoff, M., Neue Bestimmungen über die Vertheilung der Dämmerungswerthe im Dispersionsspectrum des Gas- und des Sonnenlichts, *Z. Psychol. u. Physiol. Sinnesorg.*, 1902, xxix, 255.

Sheppard, S. E., The optical and sensitizing properties of the isocyanine dyes, *J. Chem. Soc.*, 1909, xcv, 15.

Trendelenburg, W., Quantitative Untersuchungen über die Bleichung des Sehpurpurs in monochromatischem Licht, *Z. Psychol. u. Physiol. Sinnesorg.*, 1905, xxxvii, 1.

Troland, L. T., Brilliance and chroma in relation to zone theories of vision, *J. Opt. Soc. Am.*, 1922, vi, 3.

Vogel, H., Ueber die Beziehungen zwischen chemischer Wirkung des Sonnenspektrums, der Absorption und anomalen Dispersion, *Ber. chem. Ges.*, 1874, vii, 976.

Weigert, F., Über einen neuen Effekt der Strahlung, *Z. Physik.*, 1920, iii, 437; Ein photochemisches Modell der Retina, *Arch. ges Physiol.*, 1921, cxc, 177.

THE COLLOIDAL BEHAVIOR OF SERUM GLOBULIN.

By DAVID I. HITCHCOCK.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, June 28, 1922.)

I.

INTRODUCTION.

The theory of the colloidal behavior of proteins developed by Loeb[1] has been shown to apply to the proteins, gelatin, casein, egg albumin, and edestin.[2] It may be recalled that this theory is based on the idea that proteins are amphoteric electrolytes, reacting stoichiometrically with acids and bases to form ionizable salts, and on Donnan's theory of membrane equilibrium.[3] The object of the present work was to find out whether the theory would explain the behavior of a serum globulin as well.

The globulin was prepared from serum which was obtained by whipping and centrifuging fresh ox blood. The serum was diluted and the globulin was precipitated near its isoelectric point by CO_2 and purified as described by Robertson.[4] The product was therefore the insoluble serum globulin or euglobulin. The isoelectric point of this globulin was found by Rona and Michaelis[5] to be at a hydrogen ion concentration of 0.36×10^{-5} (pH 5.44). 1 per cent suspensions of the present preparation in distilled water were found to have a pH of 5.41 or 5.42 at 33°C. The removal of salts was assured by a measurement of the conductivity of a 1.628 per cent suspension

[1] Loeb, J., Proteins and the theory of colloidal behavior, New York and London, 1922; *J. Gen. Physiol.*, 1918–22, i–iv.

[2] Hitchcock, D. I., *J. Gen. Physiol.*, 1921–22, iv, 597.

[3] Donnan, F. G., *Z. Elektrochem.*, 1911, xvii, 572.

[4] Robertson, T. B., The physical chemistry of the proteins, New York, London, Bombay, Calcutta, and Madras, 1918, 40.

[5] Rona, P., and Michaelis, L., *Biochem. Z.*, 1910, xxviii, 193. Michaelis, L., Die Wasserstoffionenkonzentration, Berlin, 1914, 56.

of the globulin in distilled water; the specific conductivity at 34° was found to be 1.2×10^{-5} reciprocal ohms. The globulin was kept in suspension in distilled water saturated with thymol, and was preserved in an ice box. To obtain the concentrations of globulin required for the experiments, the bottle was shaken and samples were withdrawn by a pipette. The accuracy of this method of measuring the globulin was checked by dry weight determinations on two 25 cc. samples; each was found to contain 0.407 gm. of dry globulin.

Inasmuch as this globulin preparation did not give clear solutions with either acid or alkali, it is probable that it had become partly denatured or changed in some way during the process of purification. A second lot was prepared which gave nearly clear solutions in acid or alkali. Nevertheless, the first preparation could still be used to show whether or not a protein prepared from ox serum obeyed the same laws as other proteins.

II.

Titration of Globulin with Acids and Bases.

Titration curves were obtained by measuring with the hydrogen electrode, at 33°, the pH of 1 per cent solutions of the globulin in HCl and H_3PO_4 of various concentrations, and of a 0.5 per cent solution of the globulin in H_3PO_4. The pH values were referred to 0.1 M HCl, its pH being taken as 1.036. The results are given in Table I.

In order to determine how much of the HCl was combined with the globulin, the amounts of HCl required to give the same pH to 100 cc. of water, without protein, were subtracted from the total amounts of HCl in Table I. The figures for the acid-water curve have been given in a previous paper.[6] In the case of H_3PO_4 it was pointed out in connection with the titration of edestin[2] that this method does not give the true amounts of combined acid, on account of the repression of the ionization of the weak acid H_3PO_4 by the $H_2PO_4^-$ ion from the protein phosphate. Accordingly the amounts

[6] Hitchcock, D. I., *J. Gen. Physiol.*, 1921–22, iv, 733.

TABLE I.

Titration of Globulin with Acids.

0.1M HCl in 100 cc. of 1 per cent globulin, *cc.*	0	0	0.60	0.60	1.20	1.20	2.39	2.51	4.18	4.54	6.57	6.57	8.95	8.97	11.35	11.36	16.14	16.95	22.12	29.85	37.52	61.3
pH	5.41	5.42	3.99	4.07	3.71	3.80	3.43	3.39	3.16	3.07	2.85	2.86	2.60	2.62	2.35	2.36	2.04	2.08	1.85	1.67	1.52	1.29
0.1M H_3PO_4 in 100 cc. of 1 per cent globulin, *cc.*	1.39	2.78	5.56	9.72	16.7	24.3	34.0	43.6														
pH	3.63	3.33	2.98	2.65	2.33	2.14	1.98	1.88														
0.1M H_3PO_4 in 100 cc. of 0.5 per cent globulin, *cc.*	0.69	1.39	2.78	4.16	6.94	11.11	18.05	27.8	41.7	69.4												
pH	3.67	3.41	3.12	2.89	2.60	2.37	2.15	1.99	1.84	1.67												

of combined H_3PO_4 were calculated by the equation[7] which was used in the case of edestin

$$x = \frac{kc}{h+k} - h$$

Here x = concentration of $H_2PO_4^-$ from protein phosphate (assumed to be completely ionized).

k = primary ionization constant of H_3PO_4 = 0.01.

c = total concentration of H_3PO_4.

h = concentration of H^+ = concentration of $H_2PO_4^-$ from H_3PO_4.

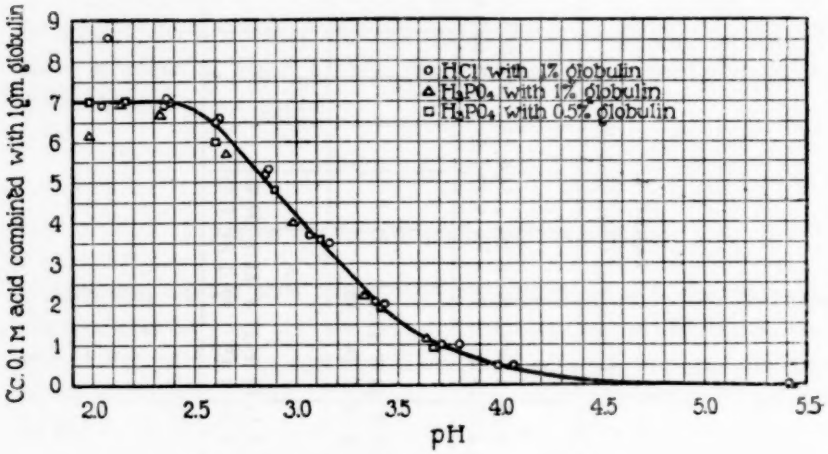

FIG. 1. Combination of globulin with acids.

The values obtained in this way, together with the values for HCl, are plotted in Fig. 1 in terms of cc. of 0.1 N acid combined with 1 gm. of globulin. Since the points fall on one curve it is to be inferred

[7] An equation identical with this was used by Pauli and Hirschfeld (Pauli, W., and Hirschfeld, M., *Biochem. Z.*, 1914, lxii, 245; Pauli, W., Kolloidchemie der Eiweisskörper, Dresden and Leipsic, 1920, pt. 1, 57) to calculate the amounts of acetic acid combined with horse serum albumin. However, since they plotted the amounts of combined acid against the total concentration of acid added instead of against the pH, they were not able to show that the protein was combined with chemically equivalent amounts of weak and strong acids.

that the globulin reacts stoichiometrically with equal numbers of molecules of the two acids. In other words, both HCl and H_3PO_4 react with globulin as monobasic acids. The combination curve appears to become horizontal at about 7 cc. of 0.1 M acid, indicating a combining weight of about 1,400 for the globulin. However, the height of the maximum is more or less uncertain. In attempting to carry the curve into the region of lower pH, it was found that the points for HCl varied irregularly on both sides of the value 7 cc.,

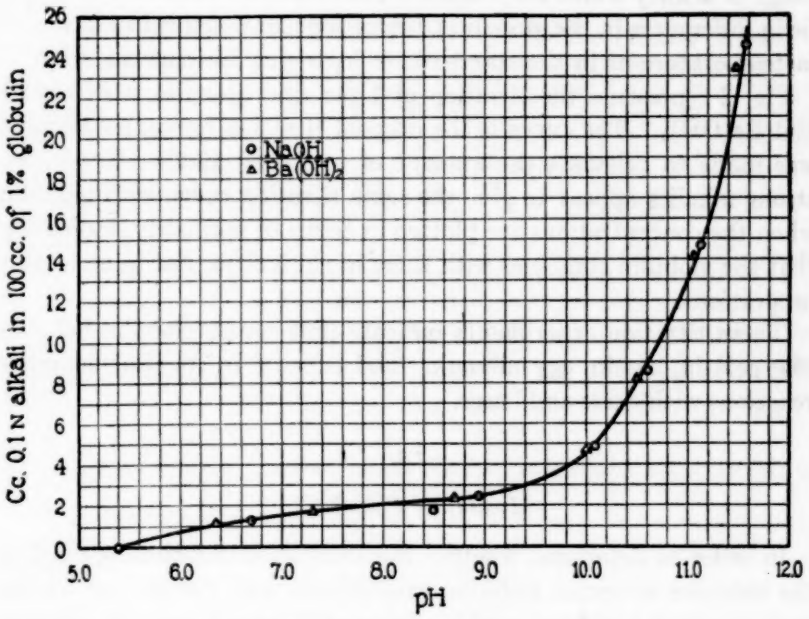

FIG. 2. Titration of globulin with alkalies.

while those for H_3PO_4 seemed to descend toward the axis of abscissæ. The former deviations are evidently due to the magnification of errors involved in taking the differences between two steep curves; the apparent decrease in the amount of H_3PO_4 combined is probably due to incomplete ionization of the globulin phosphate in the presence of much H_3PO_4, while in the calculation it was assumed that the protein phosphate was completely ionized. At any rate the results in Fig. 1 show that between pH 2 and 4 the globulin reacts stoichiometrically with equimolecular amounts of the two acids.

It should be added that a potentiometric titration of serum globulin with HCl was made by Robertson.[8] He calculated the amount of combined HCl simply by taking the difference between the total acid concentration present and the hydrogen ion concentration found in the presence of the protein. This involves the assumption of complete ionization of the HCl, which is probably correct for the concentrations he used, 0.01 M or below. His values fall at points higher than those in Fig. 1, and do not appear to reach a maximum in the range of acidity which they cover. The cause for this difference may lie in differences in the globulin preparations, or possibly in differences in temperature or in the standards of hydrogen ion concentration.

Fig. 2 represents the titration of 1 per cent globulin with NaOH and $Ba(OH)_2$. The curve is the original titration curve; no attempt was made to calculate the amount of combined alkali. These two strong alkalies appear to give the same titration curve with globulin when the concentrations are plotted in terms of normality, indicating that the globulin combines with them in equivalent, not in molecular proportions.

These titration experiments indicate that serum globulin behaves like gelatin, casein, egg albumin,[1] and edestin[2] in its stoichiometric reactions with acids and bases.

III.

Membrane Potentials.

In order to determine whether the Donnan equilibrium applied to the behavior of serum globulin, experiments were carried out by the method which had been used by Loeb[1] with gelatin and egg albumin and followed by the present writer[2] with edestin. Solutions were prepared containing 1 gm. of globulin in 100 cc. of HCl of various concentrations. These were placed in 50 cc. collodion bags fitted with rubber stoppers and manometer tubes, and the bags were suspended in beakers of HCl free from protein but of about the same pH as the protein solutions. The beakers were placed in a water bath at 25° ± 1°C., and about 20 to 24 hours were allowed for the

[8] Robertson, T. B., *J. Phys. Chem.*, 1907, xi, 437; The physical chemistry of the proteins, New York, London, Bombay, Calcutta, and Madras, 1918, 99.

attainment of equilibrium. Then the osmotic pressure was measured in terms of millimeters of the solution in the manometer tubes, and the P.D. between the inside and outside solutions was measured with the aid of saturated KCl-calomel electrodes and a Compton electrometer. The P.D. measurements were made at about 25°. The pH of the inside and outside solutions, at 33°, was then measured with the hydrogen electrode and potentiometer. The calculated P.D. values

TABLE II.

Effect of pH on P.D. *and Osmotic Pressure of 1 Per Cent Globulin Chloride.*

pH inside	4.57	4.38	3.86	3.55	3.35	3.06	2.67	2.19	1.72	1.28
pH outside	4.27	4.12	3.54	3.21	3.02	2.75	2.44	2.07	1.67	1.28
Observed P. D., *millivolts*	6.5	7.0	16.5	21.0	21.0	20.0	15.0	10.5	4.0	3.0
Calculated P.D., *millivolts*	17.5	15.0	19.0	20.0	20.0	18.0	13.5	7.5	3.0	0
Osmotic pressure, *mm.*	9	12	23	38	57	73	71	48	27	45

Effect of pH on P.D. *and Osmotic Pressure of 0.5 Per Cent Globulin Phosphate.*

pH inside	3.57	3.26	2.95	2.76	2.50	2.30	2.09	1.97	1.83	1.66
pH outside	3.32	3.00	2.70	2.54	2.35	2.19	2.02	1.90	1.78	1.64
Observed P.D., *millivolts*	12.0	13.0	13.0	11.5	9.0	7.0	5.0	4.0	3.5	2.5
Calculated P.D., *millivolts*	15.0	16.0	14.5	13.0	8.5	7.0	4.5	4.0	3.0	1.5
Osmotic pressure, *mm.*	9	16	29	33	25	22	19	18	16	14

Effect of pH on P.D. *and Osmotic Pressure of 1 Per Cent Globulin Acetate in 0.01* M *Sodium Acetate.*

pH inside	4.63	4.34	4.02	3.69	3.35					
pH outside	4.61	4.31	4.01	3.63	3.31					
Observed P.D., *millivolts*	1.0	1.5	2.0	2.0	3.0					
Calculated P.D., *millivolts*	1.0	2.0	1.0	4.0	2.0					
Osmotic pressure, *mm.*	6	6	7	9	11					

represent the differences between the E.M.F. readings obtained for the inside and outside solutions with the hydrogen electrode, corrected to 25°; in other words, the calculated P.D. values are calculated from the measurements of the hydrogen ion concentration according to the formula deduced by Donnan (which is identical with Nernst's formula).

The results of experiments on the effect of acidity on the P.D. are given in Table II.

The results show that with protein and acid alone the agreement between the observed and calculated values for the P.D. is fairly good except in the region near the isoelectric point, and the last experiment in Table II shows that agreement may be obtained in this range of pH also by the use of buffered solutions.[9]

The effect of salt concentration on the P.D. was tested by a few experiments with NaCl, $CaCl_2$, and $LaCl_3$, and the results are given in Table III. Again the agreement between the observed and calcu-

TABLE III.

Effect of Different Chlorides on the P.D. *and Osmotic Pressure of 1 Per Cent Globulin Chloride.*

Concentration of salt.	0	N/1,024			N/256			N/32		
Salt		NaCl	$CaCl_2$	$LaCl_3$	NaCl	$CaCl_2$	$LaCl_3$	NaCl	$CaCl_2$	$LaCl_3$
pH inside	3.68	3.58	3.60	3.55	3.82	4.10	3.80	3.66	3.69	3.57
pH outside	3.34	3.36	3.38	3.33	3.76	3.93	3.73	3.63	3.63	3.54
Observed P.D., *millivolts*	21.0	13.5	12.5	13.0	4.0	3.0	3.5	1.0	1.0	1.0
Calculated P.D., *millivolts*	20.5	13.0	13.0	12.5	4.0	10.0	4.0	1.5	3.5	1.5
Osmotic pressure, *mm.*	52	25	26	28	20	16	19	4	4	4

TABLE IV.

Effect of pH on the P.D. *and Osmotic Pressure of 1 Per Cent Sodium Globulinate.*

pH inside	9.20	9.98	10.47	11.05
pH outside	9.81	10.34	10.85	11.31
Observed P.D., *millivolts*	−32.0	−23.0	−18.0	−11.5
Calculated P.D., *millivolts*	−36.5	−21.0	−22.5	−16.0
Osmotic pressure, *mm.*	220	174	165	152

lated values for the P.D. is sufficient to show that the P.D. is due to the Donnan equilibrium. Moreover, the different chlorides, at equivalent chloride ion concentrations, have identical effects in depressing the P.D. This proves that here too, as in the case of other proteins, the P.D. is affected only by the ion of opposite charge to that of the protein ion.

Table IV gives results which indicate that the predictions of the Donnan theory are fulfilled on the alkaline side of the isoelectric

[9] Loeb, J., *J. Gen. Physiol.*, 1921–22, iv, 617.

point. Here the pH inside was found to be less than the pH outside, and the observed P.D. was opposite in sign to that observed with the acid solutions, the inside solution now being negative with respect to the outside. While the quantitative agreement between the observed and calculated values is not so good as on the acid side, this is probably due to the effect of CO_2 from the air on the pH values. The solutions were protected by soda lime tubes while osmotic equilibrium was being reached, but were open to the air during the P.D. measurements.

IV.

Osmotic Pressure.

The osmotic pressure of these solutions was affected in the same sense by changes in pH as in the case of other proteins. On the acid side the osmotic pressure increased from small values near the isoelectric point to a maximum in the neighborhood of pH 3, and decreased with further increases in acidity. This is qualitatively in accord with the Donnan theory. The actual calculation of the osmotic pressure from the hydrogen ion measurements, however, gave values which were much higher than those observed, the maximum in the case of globulin chloride being over 3.5 times as high as that observed. The explanation for this discrepancy may lie in the existence of large aggregates in the globulin solutions. Loeb has shown[10] that the presence of undissolved particles of gelatin in place of dissolved gelatin has very little effect on the P.D. of a gelatin chloride solution, but that it materially decreases the osmotic pressure, the undissolved particles appearing to have but a slight share in the osmotic pressure as measured by the manometer. Since many of the globulin solutions were very opaque it is quite probable that much of the globulin was in the form of particles which produced no measurable osmotic pressure. However, a second preparation of globulin gave nearly clear solutions in HCl which still had an osmotic pressure much less than that calculated from the pH measurements.

The small osmotic pressure which was measured, however, was affected by salts in the way predicted by the theory. Table III shows that equivalent concentrations of different chlorides had the

[10] Loeb, J., *J. Gen. Physiol.*, 1921–22, iv, 769.

same effect in decreasing this osmotic pressure, the decrease being evidently dependent on the concentration of the ion of opposite sign of charge to that of the protein ion.

The osmotic pressure observed in the experiments with alkali was more nearly of the same magnitude as that calculated on the basis of the Donnan theory, indicating that the sodium globulinate probably contained fewer large aggregates than the globulin chloride. Quantitatively, however, the agreement was poor; possibly this may be due to the presence of sodium carbonate.

SUMMARY.

1. The globulin prepared from ox serum by dilution and precipitation with carbon dioxide has been found, by electrometric titration experiments, to behave like an amphoteric electrolyte, reacting stoichiometrically with acids and bases.

2. The potential difference developed between a solution of globulin chloride, phosphate, or acetate and a solution of the corresponding acid, free from protein, separated from the globulin by a collodion membrane, was found to be influenced by hydrogen ion concentration and salt concentration in the way predicted by Donnan's theory of membrane equilibrium. In experiments with sodium globulinate and sodium hydroxide it was found that the potential difference could be similarly explained.

3. The osmotic pressure of such solutions could be qualitatively accounted for by the Donnan theory, but exhibited a discrepancy which is explicable by analogy with certain experiments of Loeb on gelatin.

4. The application of Loeb's theory of colloidal behavior, which had previously been found to hold in the case of gelatin, casein, egg albumin, and edestin, has thus been extended to another protein, serum globulin.

The writer's thanks are due to Dr. Jacques Loeb for his suggestion and guidance of this work.

THE EXCRETION OF CARBON DIOXIDE BY RELAXED AND CONTRACTED SEA ANEMONES.

By G. H. PARKER.

(*From the Zoological Laboratory of Harvard University.*)

(Received for publication, April 15, 1922.)

I.

INTRODUCTION.

It is well known that clams, oysters, and other invertebrates, provided with smooth muscle may contract and remain so even against considerable resistance for days or even weeks at a time. Oysters are said to keep their valves closed against the action of the ligament for from 20 to 30 days. Sea anemones are in this respect almost equally remarkable, for they have been known to remain contracted for days or even weeks.

As early as 1878 Coutance pointed out that the adductor muscles of the pelecypods were composed of two parts, one of smooth fibers, and the other of cross-striped. The quick closing of the valves was accomplished by the cross-striped muscle, and the continued closure was maintained by the smooth muscle. Marceau (1909) compared the action of these muscles to that of a cog-wheel with a catch. In such a mechanism energy was required to move the wheel, but by means of the catch the wheel could be held in any position without further work. Essentially, the same view was advanced by von Uexküll (1909) who found that among other invertebrate structures the spines of sea urchins were provided with two classes of muscles, one for motion, and the other for holding. Muscles which have this peculiar capacity of holding have been called tonus muscles and Bethe (1903, 1911), as a result of his study of snails and of clams, declared that in tonus muscle energy is consumed in passing from one state to another but not in maintaining a given state. The body muscles of the large marine snail *Aplysia* remained contracted 10 days in the

animal without food, a condition inconceivable on the assumption that contraction implies a characteristically high rate of metabolism. A similar conclusion was drawn by Bethe from the fact that fresh water mussels remained contracted under load for as long as 25 days and yet the substance of these animals was no more diminished than that of those that were normally resting. Parnas (1910), who studied the absorption of oxygen and the excretion of carbon dioxide in clams and other pelecypods, found that at maximum contraction and under high load the adductor muscles of these animals showed no increase of metabolism. Hence, the experimental evidence, so far as it goes, favors the view that in the so called tonus muscles the energy-consuming process is merely that of changing from one state of shortening or elongation to another, and that the maintenance of a state once reached is not at the expense of energy, but is dependent upon some such mechanical device as would be represented by a hook or a catch in such a mechanism as a cog-wheel (Bayliss, 1915).

The muscles of sea anemones, so far as contraction is concerned, exhibit all the properties of the so called tonus muscles. The common sea anemone of the New England coast, *Metridium marginatum* Edw., may be said to average about 6 to 8 cm. in height and 3 to 4 cm. in diameter. Such an animal in a fully expanded condition may have a volume of about 85 cc. On passing into full contraction it will discharge sea water to the extent of about 70 cc. and the residual 15 cc., representing for the most part the living substance of the animal, may remain in a contracted, compact form, for days at a time. The muscles concerned with this contraction, which are all of a very primitive, smooth variety, may shorten to as much as one-tenth their greatest length and remain persistently in this shortened state. Sea anemones afford, therefore, very convenient material for testing the question of the relative activity of tonus muscles at various stages of contraction.

As previous workers have shown, the most convenient line of attack on this problem is the metabolic one, and of the evidences of metabolism the excretion of carbon dioxide is perhaps the easiest one to determine. The question resolves itself as follows: In a given sea anemone what is the output of carbon dioxide during the four states of relaxation, of contraction, of passing into the relaxed state, and

of passing into the contracted state? Assuming that the amount of carbon dioxide excreted is a measure of the metabolism of the sea anemone, these four determinations ought to throw light on the nature of the activity of tonus muscle, for the contraction and relaxation of a sea anemone is the contraction and relaxation of its smooth muscles.

In making these determinations an Osterhout respiratory apparatus was employed. This apparatus has already been described and figured (Osterhout, 1918), and the method by which it was calibrated for this particular piece of work has already been published (Parker, 1922). Suffice it to say that the apparatus consists of a system of chambers and tubes whereby a closed circulation of air is maintained by means of a rubber pump provided with valves. From a chamber in which the organism is contained, and consequently in which the carbon dioxide is produced, the air is carried either directly to a tube filled with an indicator solution, or indirectly to it, in that on the way to the indicator the air is passed through a U-tube filled with fragments of sodium hydroxide for the absorption of carbon dioxide. In the first instance, the air, carrying a definite proportion of carbon dioxide, bubbles through the indicator and gradually charges it with carbon dioxide, thus changing its tint; in the second, the air, freed from carbon dioxide, bubbles through the indicator and washes out the contained carbon dioxide, thus returning the indicator to its original tint. Whether the air will go directly or indirectly from the chamber in which the organism is kept to the indicator, is arranged by a system of pinch-cocks. From the indicator the air is returned through the rubber pump to the chamber in which the organism is kept. In taking readings with this apparatus, a record is made of the time in seconds necessary to change the color of the indicator by means of the carbon dioxide given out by the organisms from one standard tint to another. The indicator used in these tests was an aqueous solution of phenolsulfonephthalein, and the two standard tints were those represented by pH 7.36 and pH 7.78 (see Osterhout and Haas, 1918). In taking a reading, the apparatus was run with an appropriate current of air till the indicator corresponded in tint to that of the standard tube pH 7.78; the air direct from the organism was then passed through the indicator and the time necessary to change the

indicator from the tint characteristic for pH 7.78 to that for pH 7.36 was recorded. This rate of change was assumed to correspond to the rate at which the sea anemone gave out carbon dioxide, and was the desired figure. As these readings could be made every few minutes, for it takes only a short time to set and reset the apparatus, an almost continuous record of the carbon dioxide output of the sea anemone could be kept, a great advantage which the Osterhout apparatus has over those of other types of construction. As none of the previous workers on this problem had used methods presenting this advantage, it was hoped that novel results would be obtained thereby.

The first trials were made on sea anemones in expanded or contracted states in sea water, but it was soon evident that the carbon dioxide liberated by the sea anemone was not freely given up to the air by the sea water which, without doubt, acted as a buffer solution. Fortunately, however, *Metridium* is a sea anemone that naturally spends much time between tides and consequently out of water. Therefore, it could be suspended in the air without harm, and when tested in this position, its output of carbon dioxide was found to be continuous and relatively uniform.

The work was done at the Marine Biological Laboratory at Woods Hole, Mass., and I am under obligations to Professor F. R. Lillie, director of the laboratory, and his staff for many privileges and courtesies. I am also under obligations to Professor W. J. V. Osterhout for much help and advice in the use of his respiratoy apparatus.

II.

OBSERVATIONS.

A. Relaxed Sea Anemones.—In measuring the amount of carbon dioxide excreted by relaxed specimens of *Metridium*, it was found best to hang the animal in the respiratory chamber rather than to allow it to lie on the floor of the chamber. By puncturing the foot of the sea anemone a cord could be passed inward through its mouth and out through its foot in such a way as to make a convenient loop by which the animal could be suspended from a hook in the rubber stopper of the respiratory chamber. Such a sea anemone hung like a mass of tough jelly in contact on all sides with the air of the

respiratory chamber. Occasionally the weight of the animal caused the cord to cut through the tissues and the whole animal fell to the bottom of the chamber, but in most instances the tissues were tough enough to resist the cord and the animal remained suspended during the test. The tissues of the sea anemone were too delicate to admit of much additional weighting so that the animals carried only their

TABLE I.

Times in seconds necessary to change the indicator solution in an Osterhout apparatus from pH 7.78 to pH 7.36 by the carbon dioxide excreted by relaxed specimens of *Metridium*, Nos. 1 and 2 separately, and 1 and 2 together. At the bottom of the table are given the averages of the times, the carbon dioxide excreted in hundred-thousandths of a milligram per second, the weight of the sea anemones in grams, and the weight of carbon dioxide in hundred-thousandths of a milligram secreted per second per gram weight of sea anemone.

No. of individual	1	2	1 and 2
Respiratory time, *sec.*	484	461	207
	372	438	209
	466	392	231
	382	401	190
	420	410	228
Average time	424.8	420.4	213
CO_2 per sec., *in 1/100,000 mg.*	3.0+	3.1−	6.0+
Weight, *gm.*	0.5	0.5	1.0
CO_2 per sec., per gm	6.0+	6.1+	6.0+

own weight, and the muscles in contracting lifted that weight and no more. Sea anemones that had been thus hung soon discharged most of the sea water contained in their bodies, and tests were not made till this superfluous fluid had all been drained off. The sea anemone then hung as an elongated jelly-like mass surrounded on all sides by the air of the respiratory chamber, and in this relaxed condition observations were made on the rate at which it excreted carbon dioxide.

In some preliminary trials two relaxed sea anemones of the same weight, 0.5 gm., were tested separately and together with results

as shown in Table I. The average time necessary for the appropriate change in the indicator when the two animals were tested separately was approximately the same, 424.8 seconds for No. 1 and 420.4 seconds for No. 2. Each sea anemone must, therefore, have produced carbon dioxide at about the same rate. What this rate was in absolute terms can be ascertained by using the formula obtained from the calibration of the apparatus as already referred to (Parker, 1922). This formula is as follows:

$$\left(\frac{K}{T} = W\right)$$

in which K is the constant of the apparatus, in this particular instance 1,283.5, T is the time in seconds for the indicator change, and W is the weight of carbon dioxide delivered in hundred-thousandths of a milligram per second.

In individual No. 1, this rate proved to be 3.0+ and in No. 2, 3.1− hundred-thousandths of a milligram of carbon dioxide per second. When the two animals were tested together, the average time necessary to change the indicator was found to be 213 seconds which corresponds to the production of 6.0+ hundred-thousandths of a milligram of carbon dioxide per second. Thus, the two sea anemones together made the requisite change in the indicator in almost exactly half the time taken by either alone, and gave evidence of excreting carbon dioxide at double the rate of the separate individuals. These tests not only yield results of importance in the respiration of *Metridium*, but they also indicate the relative accuracy of the apparatus.

Other relaxed sea anemones were tested for their production of carbon dioxide with the results shown in Table II. Here it will be seen that the heavier the specimen, the shorter the respiratory time, and consequently the larger the calculated amount of carbon dioxide excreted in unit time. At the bottom of the table the amount of carbon dioxide in hundred-thousandths of a milligram excreted per second by a gram weight of sea anemone is given. This amount varies from 4.8+ to 5.9+ or, if all the results in this paper are included, from 4.4 to 6.1. The average of all these measurements is 5.43− and it may, therefore, be concluded that a relaxed *Metridium* ex-

cretes for each gram of living substance about 5.5 hundred-thousandths of a milligram of carbon dioxide per second, a figure very close to that for the clam, *Venus*, as calculated from the observations of Parnas (1910), namely, 2.3 hundred-thousandths of a milligram of carbon dioxide per second per gram of living substance.

TABLE II.

Times in seconds necessary to change the indicator solution in an Osterhout apparatus from pH 7.78 to pH 7.36 by the carbon dioxide excreted by relaxed specimen of *Metridium*. At the bottom of the table are given the averages of the times, the carbon dioxide excreted in hundred-thousandths of a milligram per second, the weight of the sea anemones in grams, and the weight of carbon dioxide in hundred-thousandths of a milligram excreted per second per gram weight of sea anemone.

No. of individual	3	4	5	6	7
Respiratory time, *sec*	204	205	89	71	38
	171	200	103	58	42
	214	202	96	59	38
	200	207	92	63	43
	214	198	100	66	40
Average time	200.6	202.4	96.0	63.4	40.2
CO_2 per sec., *in 1/100,000 mg*	6.4−	6.3+	13.4−	20.2+	31.9+
Weight, *gm.*	1.2	1.3	2.7	4.2	5.4
CO_2 per sec., per gm	5.3+	4.8+	5.0−	4.8+	5.9+

B. Contracted Sea Anemones.—When a sea anemone is suspended in an Osterhout apparatus, it quickly assumes the relaxed condition and the records given in the preceding section were taken from animals in this state. The contracted condition can be easily induced by moving the glass vessel in which the sea anemone is suspended back and forth a little, whereby the sea anemone is made to swing and strike on the sides of the vessel. This movement of the vessel can be accomplished without disturbing the rest of the apparatus in consequence of the rubber connections between the vessel and the other parts. By thus stimulating the sea anemone from time to time, it can be kept in a condition of continuous and extreme contraction.

In determining the respiration of this state, a set of readings were taken with the sea anemone relaxed, a second set with it contracted, and finally a third set, after it had again relaxed. Records from two sea anemones treated in this way are given in Table III, from which it is quite obvious that the carbon dioxide given out in the contracted state is essentially the same in amount as that excreted during relaxation. Thus in the smaller animal, No. 8, the amount excreted in

TABLE III.

Times in seconds necessary to change the indicator solution in an Osterhout apparatus from pH 7.78 to pH 7.36 by the carbon dioxide excreted by two specimens of *Metridium* first, in the relaxed condition, then contracted, and finally relaxed. The bottom of the table is arranged as in Table I.

No. of individual	8			9		
State of individual	Relaxed.	Contracted.	Relaxed.	Relaxed.	Contracted.	Relaxed.
Respiratory time, *sec.*	325	305	255	162	160	164
	386	347	304	157	164	153
	277	290	347	170	163	162
	300	319	314	160	153	170
	242	298	294	172	153	168
Average time	306.0	311.8	302.8	164.2	158.6	163.4
CO_2 per sec., *in 1/100,000 mg.*	4.2−	4.1+	4.2+	7.8+	8.1−	7.9−
Weight, *gm.*		0.8			1.3	
CO_2 per sec., per gm.	5.3−	5.2−	5.3−	6.0+	6.2+	6.0+

the initial relaxation was 4.2− hundred-thousandths of a milligram per second, in contraction 4.1+, and in the subsequent relaxation 4.2+. In the large animal, No. 9, the corresponding figures are 7.8+, 8.1−, and 7.9−. Several other sets of tests were made such as the two recorded in Table III, and in all instances it was clear that the amount of carbon dioxide excreted during contraction was not significantly different from that excreted during relaxation.

C. Contracting and Relaxing Sea Anemones.—Having found that the metabolism of a relaxed and of a contracted sea anemone, as indicated by the excretion of carbon dioxide, was essentially the same,

TABLE IV.

Times in seconds necessary to change the indicator solution in an Osterhout apparatus from pH 7.78 to pH 7.36 by the carbon dioxide excreted by four specimens of *Metridium*, Nos. 10, 11, 12, 13, at the moment of contracting, of relaxing, and during the relaxed state. The records are set down in the sequence of observation. At the right of the table are given the averages of the times, the carbon dioxide excreted in hundred-thousandths of a milligram per second, the weights of the sea anemones in grams, and the weights of carbon dioxide in hundred-thousandths of a milligram excreted per second per gram weight of sea anemone.

Individual.		Sequence of observations.													Average times.	CO_2 per sec.	Weight.	CO_2 per gm.
No.	State.	1	2	3	4	5	6	7	8	9	10	11	12	13				
															sec.	*1/100,000 mg.*	*gm.*	*1/100,000 mg.*
	Contracting		248			214	196								219.3+	5.9−		5.9−
10	Relaxing			252				290							271.0	4.7+	1	4.7+
	Relaxed	270			252				243	240					251.3−	5.1+		5.1+
	Contracting			136				198		284					206.0	6.2+		6.9−
11	Relaxing				325				390		358				357.7−	3.6−	0.9	4.0−
	Relaxed	230	241			382	295								287.0	4.5−		5.0−
	Contracting			139											139.0	9.2+		9.2+
12	Relaxing				242										242.0	5.3+	1	5.3+
	Relaxed	286	270			287	221								266.0	4.8+		4.8+
	Contracting		178		270			219		249			206		224.4	5.7+		6.3+
13	Relaxing			346		326			323		374			286	331.0	3.9−	0.9	4.3+
	Relaxed	301					382					282			321.7−	4.0−		4.4+

I next attempted to determine whether there was a change in metabolism during the operations of contracting and of relaxing. For this purpose the Osterhout respiratory apparatus is especially favorable, for it allows repeated determinations of the output of carbon dioxide to be made at very short intervals. I planned, therefore, to make a determination during a period of relaxation, then stimulate the sea anemone to contraction and make a second determination while this process was going on, a third when relaxation was in progress and a fourth after relaxation had been well established. After some trials I found that my procedure was simplified by the fact that many sea anemones after a short period of suspension showed spontaneous rhythmic contractions and such contractions were of frequent enough occurrence to make it possible to use them in taking records. Four such sets of records are shown in Table IV. In these sets it was possible to get a record while the animal was contracting, reset the apparatus, and take a second record while it was relaxing and then, before the next contraction, it was usually possible to get one or two records during relaxation. The sequence and details of these operations are given in Table IV.

It is evident from an inspection of Table IV that the absolute amount of carbon dioxide excreted during the operation of contracting in all four animals is considerably greater than that produced during the operation of relaxing or during the relaxed state itself. It is also evident that the amounts of carbon dioxide excreted during the process of relaxing and during the relaxed state itself are not significantly different. These results are somewhat more evident in the last column of Table IV in which the numbers are reduced to a single standard, the weight of carbon dioxide in hundred-thousandths of a milligram excreted per second for a gram weight of sea anemone. They are still better seen in the general averages of Table V. Here it is shown that the weight of carbon dioxide, in hundred-thousandths of a milligram per second, excreted by each gram weight of sea anemone is essentially the same during the process of relaxing (4.6−) and during the relaxed state (4.8+) and that both of these weights are much lower than that shown for the process of contracting (7.1−). In other words the operation of contracting calls forth a type of metabolism in the sea anemone about half again as intense as that

involved in the process of relaxing as well as in the relaxed state itself.

The relation of the amounts of carbon dioxide excreted by a contracting sea anemone to that produced in the relaxing or relaxed animal is well seen in the plotting in Fig. 1, in which the records from specimen No. 13, in Table IV, are repeated in graphic form. Here it

TABLE V.

Average weights of carbon dioxide in hundred-thousandths of a milligram excreted per second per gram weight by four sea anemones, Nos. 10, 11, 12, 13, during the operations of contracting, and of relaxing, and during the relaxed state. The figures in the body of this table are taken from Table IV and have been used in calculating the general averages given at the end of Table V.

State of individual.	No. of individual.				General averages.
	10	11	12	13	
	1/100,000 mg.	*1/100,000 mg.*	*1/100,000 mg.*	*1/100,000 mg.*	*1/100,000 mg.*
Contracting	5.9−	6.9−	9.2+	6.3+	7.1−
Relaxing	4.7+	4.0−	5.3+	4.3+	4.6−
Relaxed	5.1+	5.0−	4.8+	4.4+	4.8+

will be seen in a most striking way that every contraction is associated with a considerable increase in the output of carbon dioxide whereas the process of relaxing and the relaxed state remain in this respect at a relatively low and uniform level.

D. General Conclusion.—From the observations on the excretion of carbon dioxide by the sea anemone *Metridium marginatum*, it is apparent that of the four states, that of contracting, of remaining contracted, of relaxing, and of remaining relaxed, the only one that calls forth increased metabolism is that of contracting. Apparently the animal may relax, may remain relaxed or may remain contracted without changing appreciably the even course of its metabolism. A metabolic change is associated only with the contracting state and we are justified in concluding that this is the only strikingly expensive operation which the sea anemone has to perform from the standpoint of its muscular activities. Once contracted, the animal may remain in that state without calling upon a type of metabolism more intense

than that characteristic of its relaxed condition. Further, the process of relaxing seems also to involve no special change of metabolism. In normal relaxing and in the state of expansion the muscles apparently are drawn out to their extreme length by the filling of the body of the sea anemone with sea water, an operation carried out by the cilia of the animal. Since the cilia are in continuous

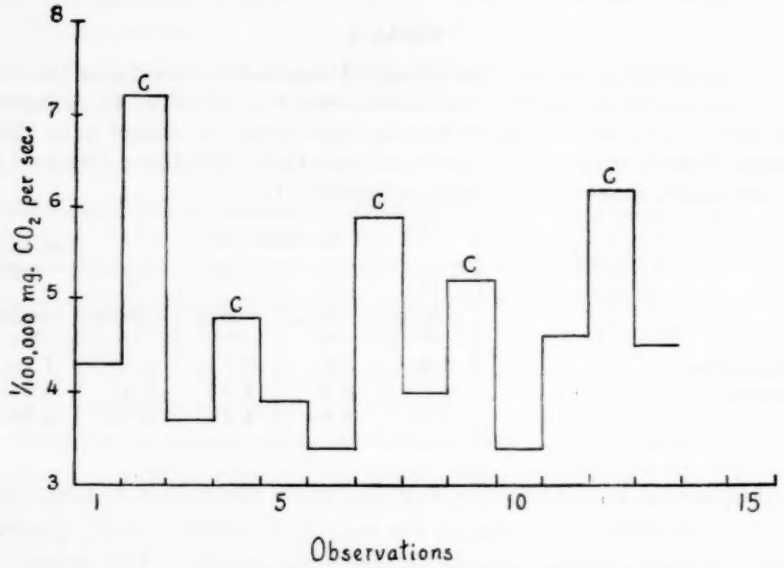

FIG. 1. Plotting of the weights of carbon dioxide in hundred-thousandths of a milligram as excreted per second by a gram weight of *Metridium* No. 13 (Table IV) during the process of contracting, of relaxing, and in the relaxed condition. The sequence of the observations is from left to right (1 to 13); weights of carbon dioxide are plotted vertically. C indicates the operation of contracting; the unmarked records represent relaxing or relaxed states.

action, the filling of the body spaces of the sea anemone with sea water, and the consequent elongation of its muscles, is an operation that involves no special activity and hence no increase of metabolism. The operation of relaxing, and the relaxed state as well as the contracted state appear, therefore, to demand no special metabolism. Contracting is the only obviously expensive operation of the four under consideration.

III.

THEORETIC.

The term tonus admits of no easy definition. Early recognized by Johannes Müller, its nervous relations were first clearly shown by Brondgeest (1860), who demonstrated in the skeletal muscles of the frog a state of slight, continuous contraction, whereby the hind legs, for instance, though quiescent, maintained a certain pose. Since this pose disappeared at once on cutting the nerve to the leg, it was correctly assumed that this type of tonus had a nervous origin. Closely related to this phenomenon is that of decerebrate rigidity to which Sherrington (1898) long ago called attention. These conditions of continuous contraction in skeletal muscles find a much more striking expression, as already intimated, in many smooth muscles, and since many of these muscles are concerned with maintaining continuously a shortening condition rather than with active contraction they have been called tonus muscles. Whether the continuously shortened condition of tonus muscle and the tonic activity of skeletal muscle are the same thing or not is uncertain, but in many respects the two phenomena are strikingly alike and both appear to be in strong contrast with the normally active state of skeletal muscle ordinarily spoken of as tetanus.

Tonus and tetanus have been contrasted from a number of standpoints and, though these contrasts may prove in the end not to be in all instances fully warranted, they point clearly to fundamental differences in the two processes.

1. Tonus can be excited by a single nervous impulse after which the state of shortening produced in the muscle may remain a long time without further nervous action. Tetanus on the other hand is the result of a succession of nervous impulses and ceases almost immediately after they cease.

2. In tonus one length of fiber is as characteristic as another and there is no zero point in changing length; in tetanus the longest natural state is the resting length and the shortest the most highly active.

3. In tonus the muscle length is independent of load; in tetanus it is dependent upon load.

4. In tonus the shortened state is maintained without evidence of tiring; in tetanus the maintenance of such a state involves tiring.

5. In tonus an increase of metabolism accompanies a change of state not the persistance of a state. In tetanus it is characteristic of the contracted state as well as of the process of contracting and perhaps of relaxing.

6. Finally tonus does not seem to exemplify the all or none principle; tetanus does.

Statements have occasionally been made to the effect that tonus is a property of smooth muscle and tetanus of cross-striped muscle, but this assumed separation is certainly not justifiable. Skeletal muscles exhibit tonus and, among invertebrates at least, smooth muscle appears to contract in a tetanic way. Both processes probably occur in most muscles, but tetanus is predominantly characteristic of cross-striped muscle and tonus of smooth muscle.

The muscles of sea anemones show in their activities most of the peculiarities of the state of tonus. They can be excited to contraction by a single stimulus and they will remain in this state for a very long time without further stimulation. *Metridium* is most fully expanded and its muscle fibers consequently longest when it is in dim light, but under such circumstances it can shorten to half-length or contract completely and remain at either of these conditions almost indefinitely. Its muscle fibers, so far as length is concerned, must reproduce the state of those found by Grützner (1904) in the muscular cavities of the vertebrates in which the fibers are short when the organ is contracted and long when it is dilated, and yet they are not stretched but are as much at rest when they are short as when they are long. The fiber seems to be not unlike the tube of a telescope; it may be lengthened or shortened, but is at rest at any length.

Because of the delicacy of the tissues of *Metridium* it was difficult to work with these animals under more than the weight of their own substance. In many instances, however, animals that were hung up held up their own weight, 1 or more gm. for hours at a time, much as clams and other mollusks are known to do (Parnas, 1910; Bethe, 1911). As in the case of mollusks, sea anemones can remain contracted for hours or even days at a time without the slightest evidence of tiring. With *Metridium* there are insufficient data to

carry out a calculation to show the degree of exhaustion that would result if the muscles of this animal acted as the cross-striped muscles of other animals do. Bethe (1911) has shown that on a conservative basis the adductor muscles of a clam would have used up 8.8 times the weight of the whole clam in sugar in keeping the valves closed against the action of the ligament for the experimental period of 25 days, had they acted as cross-striped muscles. He also pointed out that the contraction of the arterioles in the mammalian circulatory system would account for at least one-sixth of the total resting metabolism of the animal on the basis of tetanic contraction. That none of these tonus organs give evidence of tiring, not to mention exhaustion, is a clear indication of the fundamental difference between tonus and tetanus.

So far as metabolism is concerned, as judged by the amount of oxygen absorbed and carbon dioxide excreted, the results of Parnas (1910) and of Bethe (1911) show quite conclusively that the shortened state of the tonus muscle, whereby, for instance, a load on the valves of a clam is resisted, calls for no more expenditure of energy than does the shortened but unloaded state of the muscle, and Bethe draws the final conclusion that in tonus muscles the change of state and not the maintenance of a state is what requires increased metabolism.

This conclusion is in large part supported by the results from *Metridium*. In this animal it is quite clear, as has already been shown, that in the relaxed and the contracted states the metabolism is essentially the same; *i.e.*, that represented by an excretion of from 5 to 6 hundred-thousandths of a milligram of carbon dioxide per second (see Table III). But at the moment of change of state the metabolism does not always necessarily change as assumed by Bethe. In *Metridium* the process of contracting is accompanied by an increase in metabolism, but that of relaxing is apparently not so accompanied. This operation calls for a type of metabolism no more intense than that of the contracted or of the relaxed state (see Table V), whereas the contracting phase is accompanied by a metabolism once and a half that of any of the other three phases. Hence, so far as *Metridium* is concerned, one is not warranted in concluding that every change of state is accompanied by a change of metabolism, for the process of contracting is the only one that shows an obvious metabolic increase.

If we assume that the muscle fibers of *Metridium* are provided with some such catch or hook mechanism as has been mentioned for tonus muscles and that the activity of these fibers is indicated by the animal's metabolism, we can then picture their action in the following way. A fully elongated fiber can be brought through nervous stimulation to any state of shortening up to approximately one-tenth its greatest length. This operation necessitates increased metabolism. The shortened form may be maintained indefinitely and without any special rise in metabolism by the catch mechanism already mentioned. On the release of this mechanism, the fiber is in a position to become fully elongated by the gradual filling of the sea anemone with sea water and the consequent extension of its parts. This operation is dependent upon cilia which are in continuous activity and which hence call forth no special rise in metabolism in causing the muscle fibers to elongate. The fully elongated fiber may remain in this state indefinitely without bringing about any exceptional rise in metabolism, a condition which is true for any length at which the fiber may temporarily be. Hence there is no zero point for the fiber. It may be at rest at any length.

In some such way as this one can picture the activity of a tonus muscle, an operation which in many respects is in strong contrast with that of a cross-striped muscle where not only the contracting phase, but the state of continued contraction, and possibly also the relaxing phase are conditions of high, often exceptionally high, metabolism. What still remains to be accounted for in the action of the tonus muscle is the catch apparatus.

This apparatus is capable of holding the contractile fibrils at any length for an indefinite period. It is, therefore, the essential mechanism of the whole tonus device. Except in the crude mechanical conception of a catch, it is difficult to picture what this device may be.

Muscle, and particularly smooth muscle, is not the only form of tissue that exhibits the kind of tonus that has just been discussed. The melanophores of vertebrates show a state very like that of the tonus of smooth muscle. The similarity of these two types of tissue has already been insisted upon by Spaeth (1916). A melanophore under appropriate stimulation may gather its pigmented substance together into a small sphere and remain so indefinitely or it may spread

out into a most extensive branch work and remain indefinitely in that state. If the nerves supplied to the melanophores in such a fish as *Fundulus* are cut, the melanophores, as has been shown by Mr. L. C. Wyman in work about to be published, assume a form with blunt processes, the stellate form, and remain indefinitely in this condition. Since any of these states may persist without limit, no one of them can be said to be the resting state. The melanophore may assume a completely expanded, a completely contracted, or a partly contracted state, and remain thus for days. The condition is exactly parallel with that of a tonus muscle and suggests the presence of a catch mechanism in the melanophore as in the muscle fiber. But such a mechanism, if present in the melanophore, can have nothing to do with myofibrils, for the melanophore contains no such elements. The mechanism must, therefore, be located in the cytoplasm of the melanophore. Granting this to be so, the place to look for the catch mechanism in the muscle fiber is, then, not in its contractile elements, the myofibrils, but in that part of it which resembles the cytoplasm of the melanophore, namely, its sarcoplasm. Here in all probability is located the mechanism which can hold the myofibrils at a definite length or release them for their characteristic changes. The catch is apparently a function of the sarcoplasm.

How the catch mechanism operates, I am at a loss to say. Possibly it may depend on a temporary coagulation of the sarcoplasm, such as Biedermann (1904, p. 523) has suggested for the muscle fiber as a whole. Thus after the shortening or lengthening of the myofibrils has occurred, the fixed state would be assumed by the passage of the sarcoplasm from a condition of sol to one of gel whereby the myofibrils would be held definitely at a given length. Only after the sarcoplasm had reversed its state from a gel to a sol would the myofibrils be again in a position to change their length, either elongating or shortening. Thus the state of the surrounding sarcoplasm would determine whether the myofibrils were to be held at a definite length or were to be free to change their length either by contracting or by being drawn out. In this way it is conceivable that the sarcoplasm might act as a catch or hook mechanism.

That some stimulus must induce the assumed changes in the sarcoplasm from one state to another and back again is perfectly evi-

dent, but what this stimulus is I do not suggest. Whatever it may be it probably involves so slight a change in the total metabolism of the muscle as to make no perceptible impression upon it. Thus it probably escapes detection. In respect to the relatively small amount of energy that may be necessary to control this change it recalls again a catch or tooth mechanism working on a cog-wheel.

The view here put forth has certain superficial resemblance to one already advanced by Bottazzi (1897) who postulated in muscle fibers two kinds of contractile substance, the anisotropic substance having to do with quick twitches such as are involved in tetanus, and the sarcoplasm concerned with slow contractions such as are seen in tonus. According to the view advanced in the present paper, however, all contraction is myofibrillar and the sarcoplasm is concerned solely with the catch mechanism. In this respect the present view is in strong contrast with that of Bottazzi. That the opinion advanced in this paper as to the method of action of the catch mechanism is highly speculative is fully appreciated.

IV.

SUMMARY.

1. The metabolism of the sea anemone *Metridium marginatum* Edw. was measured in four states, relaxed, relaxing, contracted, and contracting, by means of an Osterhout respiratory apparatus. The basis of measurement was the number of hundred-thousandths of a milligram of carbon dioxide excreted per second by a gram of living sea anemone.

2. In the relaxed state this varied from 6.1 to 4.4+ and averaged 5.43−.

3. In a comparison of the relaxed and contracted states the amount of carbon dioxide excreted was found to be about the same; in one instance in relaxation 4.2 and in contraction 4.1+; in another in relaxation 7.8+ and 7.9− and in contraction 8.1−.

4. In a comparison of the three states relaxed, relaxing, and contracting, the first two were found to average about the same, 4.8+ and 4.6− respectively and the last proved to be appreciably higher 7.1−.

5. It is, therefore, concluded that the process of relaxing and the states of relaxation and of contraction are accompanied by no unusual metabolism, but that in the operation of contracting the metabolism becomes about half again as intense as that characteristic of the other states.

6. The maintenance of the contracted state in *Metridium* for days at a time without an increase of metabolism indicates that its musculature is of the type known as tonus muscle.

7. In tonus muscle, contraction is accomplished by an active shortening of the myofibrils, extension by a passive drawing out of these fibrils through the distension of the adjacent cavities, etc., and the continued maintenance of any particular state of shortening by some form of catch mechanism in the muscle, such, possibly, as the gelation of its sarcoplasm.

BIBLIOGRAPHY.

Bayliss, W. M., 1915, Principles of general physiology, London, xx, 850 pp.

Bethe, A., 1903, Allgemeine Anatomie und Physiologie des Nervensystems, Leipsic, vii, 487 pp.

Bethe, A., 1911, Die Dauerverkürzung der Muskeln, *Arch. ges. Physiol.*, cxlii, 291

Biedermann, W., 1904, Studien zur vergleichenden Physiologie der peristaltischen Bewegungen, *Arch. ges. Physiol.*, cii, 475.

Bottazzi, F., 1897, Le oscillazioni del tono atriale negli anfibi con una teoria sulla funzione del sarcoplasma nei tessuti muscolari, *Morgagni*, xxxix, pt. 1, 535.

Brondgeest, P. Q., 1860, Ueber den Tonus der willkührlichen Muskeln, *Arch. Hollend. Beitr. Natur- u. Heilk.*, ii, 329.

Coutance, A., 1878, De l'Énergie et de la structure musculaire chez les mollusques acephales, Paris, 63 pp.

Frank, O., 1904, Thermodynamik des Muskels, *Ergebn. Physiol.*, iii, pt. 2, 348.

Grützner, P., 1904, Die glatten Muskeln, *Ergebn. Physiol.*, iii, pt. 2, 12.

Marceau, F., 1909, Recherches sur la morphologie, l'histologie et la physiologie comparées des muscles adducteurs des mollusques acéphales, *Arch. zool. exp. et gén.*, ii, series 5, 295.

Osterhout, W. J. V., 1918, A method of studying respiration, *J. Gen. Physiol.*, i, 17.

Osterhout, W. J. V., and Haas, A. R. C., 1918, A simple method of measuring photosynthesis, *Science*, xlvii, 420.

Parker, G. H., 1922, The calibration of the Osterhout respiratory apparatus for absolute quantities of carbon dioxide, *J. Gen. Physiol.*, vi, 689.

Parnas, J., 1910, Energetik glatter Muskeln, *Arch. ges. Physiol.*, cxxxiv, 441.

Sherrington, C. S., 1898, Decerebrate rigidity, and reflex coordination of movements, *J. Physiol.*, xxii, 319.

Spaeth, R. A., 1916, Evidence proving the melanophore to be a disguised type of smooth muscle cell, *J. Exp. Zool.*, xx, 193.

von Uexküll, J., 1909, Umwelt und Innenwelt der Tiere, Berlin, 259 pp.

Weinland, E., 1918, Beobachtungen über den Gaswechsel von Anodonta cygnea L, *Z. Biol.*, lxix, 1.

CELL PENETRATION BY ACIDS.

VI. The Chloroacetic Acids.

By W. J. CROZIER.

(Contributions from the Bermuda Biological Station for Research, No. 140, and from the Zoological Laboratory of Rutgers College, New Brunswick.)

(Received for publication, April 10, 1922.)

1. Method.

Several previous communications have provided measurements of the apparent rates of penetration of integumentary cells of the nudibranch *Chromodoris zebra* by different acids (Crozier, 1916 *a*, *b*). In the present report further data are given in continuance of this inquiry. The experiments were chiefly made in 1917. The work does not admit of very refined analysis, but inasmuch as the measurements are of use in related investigations (*cf.* Crozier, 1918 *b*), it is thought desirable to print them—particularly in view of the rare occurrence in animal tissues of indicators appropriate for such observations (Crozier, 1918 *c*).

Rather extensive dilution curves must be established for a number of acids, if the measurements are to be useful for discussion of the mechanism of penetration. Haas (1916) found that with solutions of external C_H. = 0.01 N, acetic acid penetrated various plant cells more quickly than did HCl, whereas several other acids, under these conditions, penetrated with equal speeds. The formal concentration of acetic acid at C_H. = 0.01 is about 4.4 N; of HCl, 0.01 N. If a similar comparison be made of two weak acids, acetic and butyric, it is found that in concentrations above 0.5 N acetic penetrates more quickly than does butyric (*cf.* Fig. 2), but in more dilute solutions the weaker acid, butyric, penetrates much more easily. At the point of intersection of the respective curves, the C_H. is higher with the acetic solution (2.55×10^{-3}) than with the butyric (2.1×10^{-3}). At the highest concentrations used, 1.0 N butyric penetrates in the same time

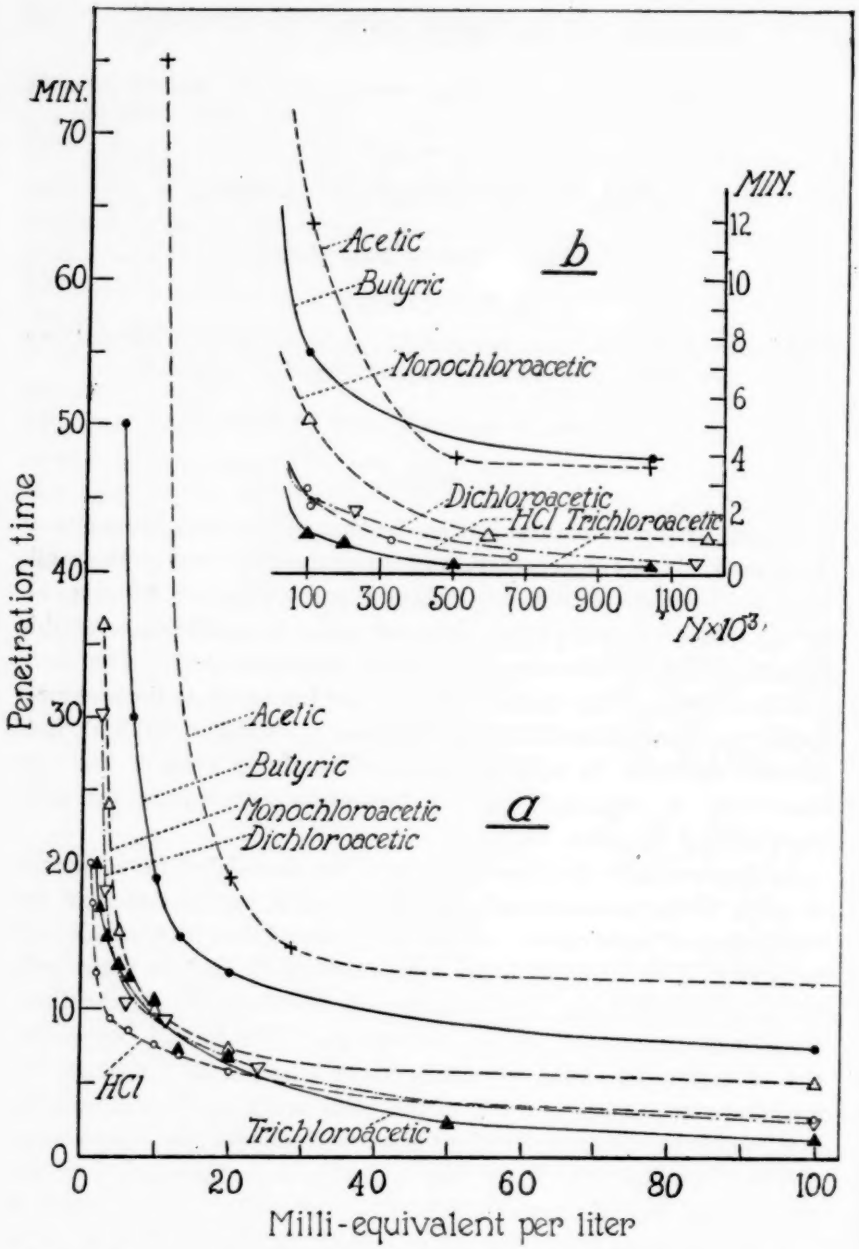

FIG. 1. *Abscissæ*, concentrations of acid, N×10−3; *ordinates*, penetration times, minutes. Each plotted point is the mean of a number of concordant readings. The zone of highest concentrations (*b*) and the lower range of concentrations (*a*) drawn separately, for clearness.

as 0.50 N acetic (3.9 min., at 27°), the C_H. being about the same in each case, 3×10^{-3} N. If comparisons were made only at this concentration, then, it might be stated that from solutions of equal pH these two acids penetrate cells within the same time; yet in all weaker solutions of corresponding pH values in the two instances, butyric penetrates more readily than does acetic (Fig. 1). These relations persist when a correction is applied taking into account the amount of acid required to produce the internal color change (Crozier, 1918 *b*). The relative effect of the hydrogen ion concentration on the speed of penetration is augmented with increasing concentration of acid; similar considerations apply to the comparison of HCl with acetic acid. Clearly, there are aspects of the penetration process, as observed in these experiments, which require for the analysis data pertaining to a range of concentrations.

Interpretation is facilitated, furthermore, by comparison of the acids within single chemical groups (Crozier, 1916 *a*). The chloroacetic acids were therefore used, in conjunction with redeterminations of penetration curves for acetic and for hydrochloric acids.

The measurements plotted in Fig. 1 were secured according to the method outlined in an earlier paper (Crozier, 1916 *a*). It is necessary to again refer to certain aspects of the procedure.

The nudibranchs used were freshly collected individuals of nearly uniform size, and as nearly as possible of the same type of pigmentation. Strips were cut from the margin of the mantle-fold, which is conveniently erected as a result of handling the animal. Fig. 3, a cross-section in the mid-body region of a moderately contracted individual, indicates at 1.....1 the approximate line of cutting. From such strips pieces 1 cm. long were cut. A bit of mantle tissue so prepared is sketched at *B* of Figure 3, with its cross-section shown at *C*. Remaining in sea water for 10 to 15 minutes, the pieces were then wiped gently on filter paper, and placed in an acid solution. Pieces so prepared lived for a week in sea water. During several days they tend to show about the same resistance to the penetration of acid, but they more easily give up their intracellular pigment; the mantle is ciliated, and the tissue fragments therefore travel for some days over the bottom of the dish, so the vitality of a fragment is readily ascertained.

When a fragment of the mantle is placed in acid, a cut surface is presented for diffusion. It might be supposed that acid migrates through the spongy tissue laid open by the cutting, and that the observed color change depended upon this diffusion rather than upon penetration through the outer epithelial surface. I have already called attention to the fact that this idea would be erroneous (Crozier,

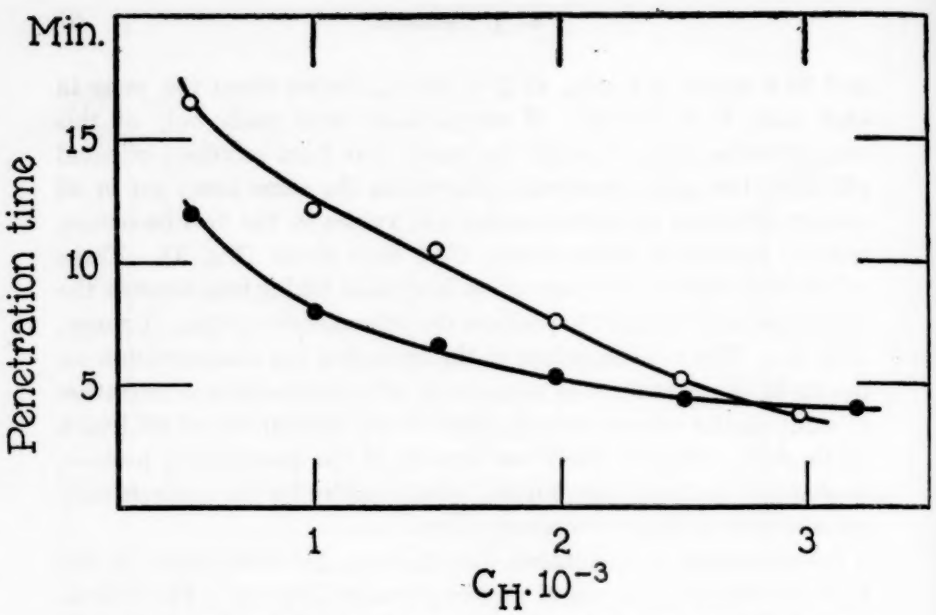

FIG. 2. Penetration of acetic acid (upper curve) and of butyric acid (lower curve) from solutions of corresponding pH.

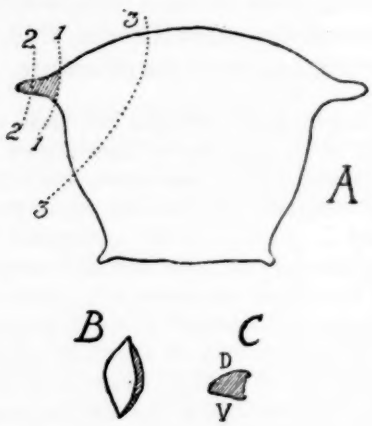

FIG. 3. *A*, Cross-section of *Chromodoris zebra* in the mid-body region; 1....1, line of amputation in preparing pieces of the mantle fold for penetration tests. Pieces removed at this depth give readings corresponding to those with pieces cut at the level 3....3, or with the entire nudibranch. Pieces removed at the level 2....2, present too great a proportion of damaged tissue, and penetration is very rapid in all cases. *B*, an isolated tissue fragment used in penetration tests, and *C*, its cross-section.

1916 *a*, page 263), but further experimental proof of this seems worth citing. Unless very small fragments of the tissue are used, 2 to 3 mm. broad, in which case there is usually a relatively considerable proportion of tissue mechanically injured in the zone of cutting, the penetration time of an acid solution is found to be independent of the size of the mantle fragment; it is identical with that obtained when the uncut whole nudibranch is immersed in the solution, provided the solution be stirred, and provided also that a sufficiently large volume of acid be used. The penetration, therefore, takes place through the outer surface of the mantle epithelium. The tests were made under such conditions—namely, with a large volume of solution—that the concentration of the external medium may be regarded as essentially constant. The results are therefore more simple to interpret than are findings such as those reported by Miss Hind (1916), where variations in the external concentration, only in part due to absorption of acid, form, in fact, the basis of measurement.

2. *Relation to Size of Animal.*

Mention has earlier been made of the fact that the penetrability of mantle tissue of *Chromodoris zebra* by acids is more rapid in the case of the smaller individuals (Crozier, 1916 *a*). It should be important to decide whether this condition may have reference, primarily, to a decreased permeability of the outer surfaces of the cells in the older individuals, or rather to a general toughening of the protoplasm as a whole as age progresses. Particularly, it would be most desirable to secure a *measure* of the relationship between size, (age) and resistance to penetration.

Observations secured with HCl in a preliminary study of this matter are given in Fig. 4. The measurements, made in 1917, were carried out with due attention to such precautions as have previously been noted (Crozier, 1916 *a*; 1918 *b*). A few experiments with other acids (HNO_3, salicylic) showed that the greater apparent speed of penetration in the case of tissue from the smaller animals is quite general.

The regularity of the curves drawn invites their closer analysis. This cannot be carried very far, because in the existing data it is not possible to correct the observations according to the minimal concentration of acid which for each particular size of individual is required to effect the intracellular color change (Crozier, 1918 *b*); presumably, this correcting factor changes with increasing age. Nevertheless it is possible to arrive at a first approximation to a factor

giving a measure of the resistance offered by the tissue toward the inward diffusion of acid.

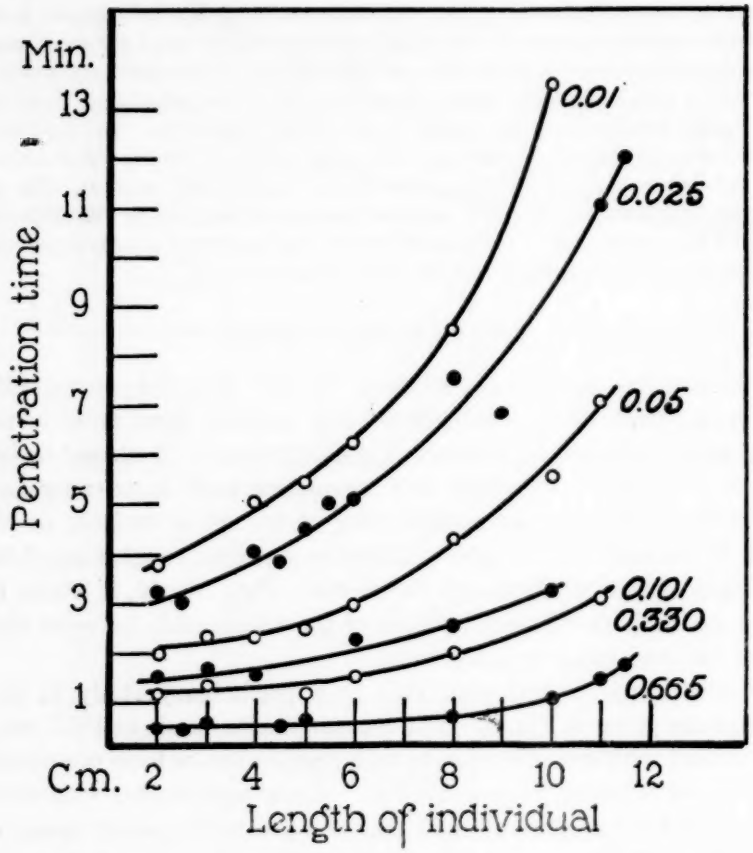

FIG. 4. Each curve shows the relation, for that concentration of HCl, between *observed time of penetration* (minutes), and *the size of individual* (cm.) from which the tissue had been obtained; each point represents the mean of a number of readings.

An equation has been suggested by Stiles (1920) which empirically satisfies the results of his experiments upon the diffusion of NaCl into agar gels containing $AgNO_3$. The equation has the form

$$\frac{p}{\sqrt{t}} = K \log C + K' \qquad (1)$$

in which t signifies the time elapsed for a distance of penetration p from a solution of concentration C; K' is a constant, varying chiefly with the nature of the penetrating salt; and K is a function chiefly of the concentration of $AgNO_3$ in the agar, constant for a given concentration of $AgNO_3$, but decreasing with an increase in this concentration.

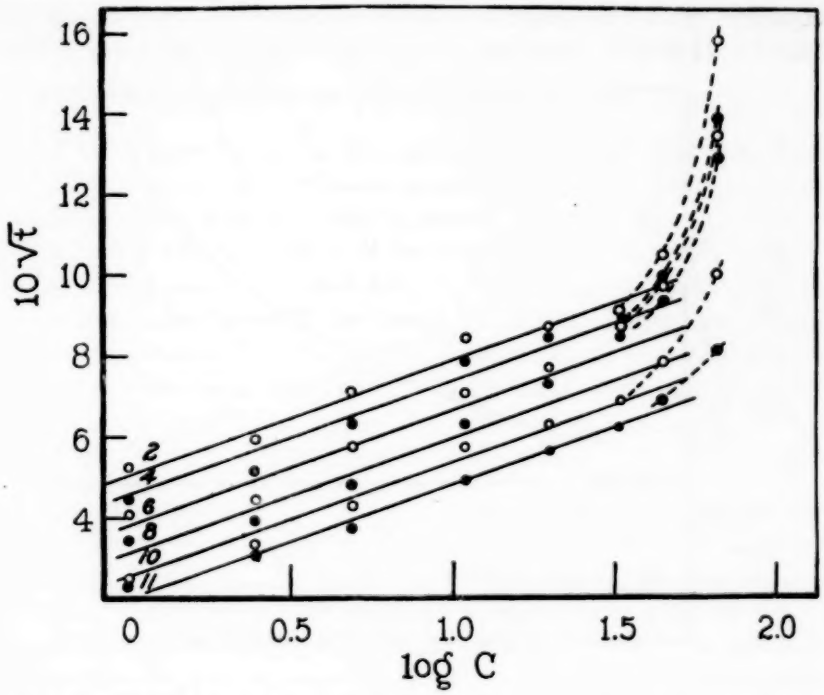

FIG. 5. To show that within a rather wide zone of concentrations the square root of the velocity of penetration (ordinate) is directly proportional to the logarithm of the concentration (abscissa); and that the graphs for the various sizes of individuals (2 to 11 cm.) are sensibly parallel within this zone of concentrations.

It may be assumed that in the *Chromodoris* tissue the distance p—to which there must have penetrated an amount of acid adequate to occasion the indicative color change—is essentially a constant quantity; Conklin (1912) has shown, in mollusks, that cell size is not a function of body size, but is constant during by far the greater part of the life duration. There is no evidence of age changes in the

distribution of the *Chromodoris* pigment. Under these circumstances, we have then

$$\frac{1}{\sqrt{t}} = k \log C + k' \qquad (2)$$

In Fig. 5 the observations already given are plotted in the form of equation (2). The points are taken from the smoother curves of Fig. 1. It is seen that the straight line equation (2) gives a fair

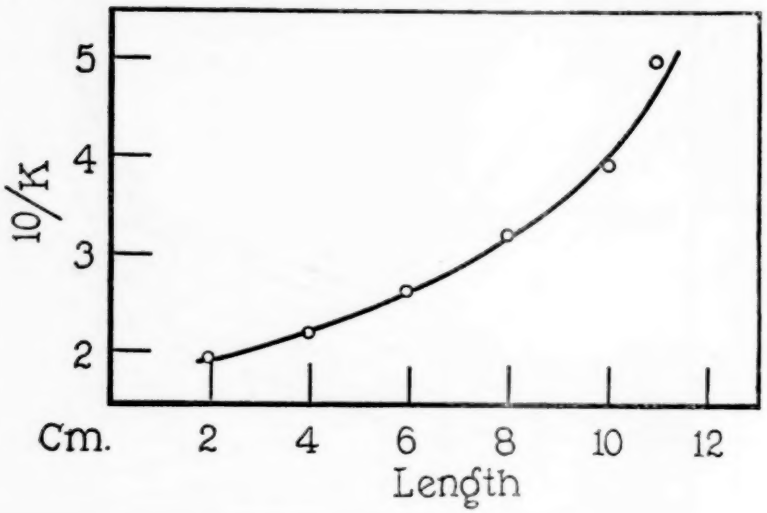

FIG. 6. Variation of $\frac{1}{k'}$ with size of individual.

account of the relation between size of individual and the penetration speed of each concentration of HCl. The deviations of the higher concentrations of acid from the straight line rule are due to the preponderating participation here of simple diffusion phenomena following rapid coagulation (Croizer, 1918 *b*), whereas with lower concentrations, the continuous combination of acid with protoplasm provides the slowest changes entering into the determination of the observed penetration time.

The straight lines in Fig. 5 are parallel, corresponding to the nature of the constant K in Stiles' experiments, whereas k' varies inversely

with the penetrability of the tissue. The reciprocal of k' may therefore be suggested as an appropriate measure of the relative resistance of the tissue toward diffusion of acid from the exterior. The magnitude of this reciprocal varies smoothly with the size of the individual, as shown in Fig. 6. Even taking into account the presumably higher "titration correction" applying to tissue from larger individuals, it may be argued from Fig. 6 that during growth the penetrability of the tissue decreases more rapidly than the length of animal increases.

Little can at present be said as to the physical meaning of $\frac{1}{K'}$. It is clear, however (from the analogy with the salt diffusion), that the resistance of the protoplasm as a whole increases with increasing size; data from growth studies in general suggests that this may in part be due to a diminution in the proportion of intracellular water. Conceivably, surface permeability varies in a parallel way. If this be the case, one factor in the lower excitability of larger individuals by acid solutions (Crozier, 1918 *a*) must reside in the tougher consistency of the superficial layer of the receptive elements.

3. Relation to Temperature.

The penetration of HCl from each of several concentrations was determined over a range of temperatures. The measurements are collected in Fig. 7. Solutions more dilute than N/160 were not employed, because with such solutions it would have been necessary to make a special study of correction, factors involving the minimal quantities of acid required at lower temperatures to effect color change in the tissue. In concentrations of HCl greater than N/100, these factors are relatively insignificant. Animals 8 to 11 cm. long were used throughout the experiments. Each of the plotted points (Fig. 7) represents the mean of several concordant measurements.

The values of the temperature coefficients exhibit some interesting variations. In Fig. 8, *temperature* is plotted against the *logarithm of the penetration velocity*. For the highest concentration used, 0.33 N, the graph is linear beyond 18°C., with Q_{10} constantly 1.89; for 0.101 N acid, $Q_{10} = 1.81$; 0.01 N, $Q_{10} = 1.68$; 0.0063 N, $Q_{10} = 1.19$. This series shows that with decreasing concentration some factor of

a purely physical character enters in a preponderating manner, especially below 0.01 N acid, into the determination of the observed result. The highest concentration provides a temperature coefficient of magnitude demonstrating that here some chemical event is probably the slowest in the series of changes resulting in observed penetration.

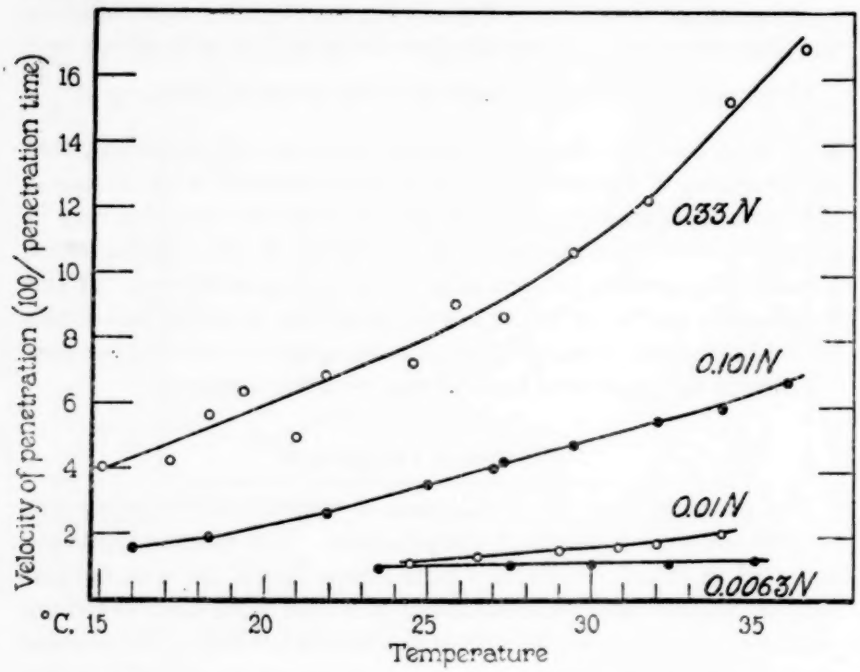

FIG. 7. Penetration of *Chromodoris* tissue by several concentrations of HCl, at different temperatures. The temperature range for normal activity of the whole animal is about 12–37°. Each curve concerns a single (indicated) concentration of HCl.

Osterhout (1914; 1917) has pointed out that the temperature coefficient of the electrical resistance of protoplasm is of the order 1.33, and that higher temperature coefficients obtained in different ways by other workers cannot be held to apply directly to the phenomenon of *permeability*. The present experiments are taken to signify that in the penetration of acid from dilute solution the observed velocity

of penetration is controlled chiefly by physical conditions having to do with the rate of diffusion, but that the initial event is a combination, of chemical nature, between acid and superficial protoplasm. This initial event is to be clearly observed only in rather concentrated solutions; its presence in more dilute solutions is inferential, but is strongly supported by the behavior of the chloroacetic acids, subsequently described.

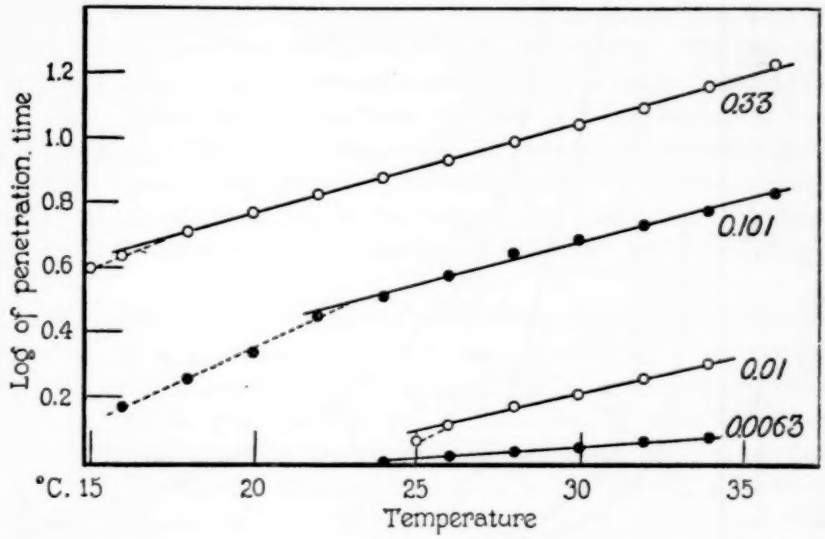

FIG. 8. As in Fig. 1, each curve pertains to a single concentration of HCl; points taken from smoothed curves in Fig. 1.

4. The Chloroacetic Acids.

The impossibility of establishing a seriation of acids, with reference to their relative penetrating ability, on the basis of observations at any single concentration, is sufficiently evident (*cf.* Fig. 1). Harvey (1914) found all three chloroacetic acids to penetrate tissue within about the same time, from 0.01 N solutions; this is also the case in my measurements, although the relative position of the three acids is further down in the list (at this concentration) than in the case of the tissue used by Harvey, and also (judging by the position of trichloroacetic) than for the plant cells used by Haas (1916).

Inspection of each penetration curve shows that beyond a certain ("critical") concentration the speed of penetration rapidly increases. The existence of a parallelism between the magnitudes of the respective concentrations at these critical points and the positions of the viscosity maxima in the protein-acid systems investigated by Pauli

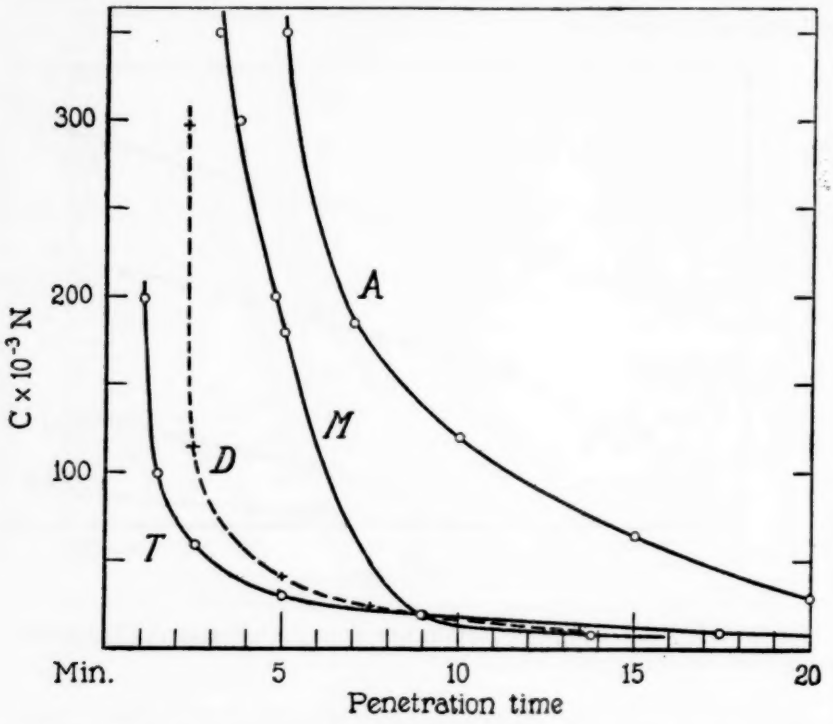

FIG. 9. Penetration time for acetic (*A*), monochloroacetic (*M*), dichloroacetic (*D*), and trichloroacetic (*T*) acids; concentrations and penetration times corrected according to the procedure described in the text. The observations extend beyond the limits of the figure. Points taken from smoothed curves in Fig. 1.

and Handovsky (1909), led to the idea that an effect upon cell proteins was primarily involved in penetration (Crozier, 1918 *b*). We now know, from Loeb's analysis of the chemical behavior of proteins (Loeb, 1920), that these viscosity maxima in fact occur at almost, if not quite exactly, the same pH in the case of all the acid-albumin

mixtures. The concentrations of the acids at the viscosity maxima state merely the relative quantities of these acids which at this pH are in equilibrium with the protein.

It may then be supposed that beyond some acid concentration corresponding to that involving a maximum viscosity of the tissue protein, the end-event in the penetration process becomes rapidly accelerated. The time intervals required to produce a maximum electrical resistance in *Laminaria* tissues immersed in a series of concentrations of HCl (Osterhout, 1914 *a*) show a good parallelism with penetration-time observations with *Chromodoris*. The velocity of penetration should in consequence depend both upon the strength of the acid and upon the readiness with which it is taken up by the protoplasm. The latter factor may vary independently of the acid strength; hence the desirability of comparing acids within groups of chemical relationship. It has been shown that in such groups, ionization determines the relative penetrating ability (Crozier, 1916 *a*),

The penetration curves for the acetic series obviously resemble, in their relative positions, the order and disposition of the curves showing the influence of these acids upon the C_H. in protein solutions (Loeb, 1920–21, page 90, Fig. 1). If it be that in order to secure corresponding speeds of penetration with different acids, a certain definite pH must be established by them in the superficial layer of the protoplasm, this constituting the "critical" event, then the members of the acetic series should arrange themselves in precisely this order. Choosing pH 3.5, corresponding, with all the acids, to about the acidity at maximum viscosity and maximum osmotic pressure (Loeb, 1920–21), as the probable critical pH, these acids should be equally efficient in the following relative concentrations (that of trichloroacetic taken as unity):

	Relative concentration.			
Acid.	Acetic.	Monochloroacetic.	Dichloroacetic.	Trichloroacetic.
pH				
4.0	7.6	1.16	1	1
3.5	10.2	1.12	1	1
3.0	26.0	1.67	1.04	1

To compare with such figures it is necessary to apply to the gross penetration data a correction which takes into account the quantity of each acid necessary to effect the intracellular titration (Crozier, 1918 *b*). This is done by subtracting from each nominal concentration the limiting concentration of that acid which is effective in penetration, and from the corresponding speed of penetration subtracting the speed of penetration of the limiting concentration. For the acetic series this has been done in Fig. 9; these curves show the ability of different concentrations of each acid to force into the tissue a quantity of that acid which is adequate to produce the indicative color change.

For comparison with the concentrations concerned in producing a definite pH in gelatin mixtures, we may choose a penetration time of about 7.5 minutes, as the absolute concentrations involved then correspond closely in the two cases (*cf.* Loeb, 1920–21):

	Relative concentration.			
Acid.	Acetic.	Monochloroacetic.	Dichloroacetic.	Trichloroacetic.
Corrected penetration time.				
5 min.	14.0	7.2	1.2	1
7.5 "	8.1	2.4	1.09	1

Qualitatively, a closer parallelism could not in the nature of the case be expected.

SUMMARY.

Measurements of the penetration of tissue from *Chromodoris zebra* are believed to show that a determining factor in penetration involves the establishment of a critical pH (near 3.5) in relation to superficial cell proteins. The rapidity with which this state is produced depends upon acid strength, and upon some property of the acid influencing the speed of absorption; hence it is necessary to compare acids within groups of chemical relationship.

The actual speed of penetration observed with any acid is dependent upon two influences: preliminary chemical combination with

the outer protoplasm, followed by diffusion. The variation of the temperature coefficient of penetration velocity with the concentration of acid, and the effect of size (age) of individual providing the tissue sample agree in demonstrating the significant part played by diffusion. In comparing different acids, however, their mode of chemical union with the protoplasm determines the general order of penetrating ability.

BIBLIOGRAPHY.

Conklin, E. G., 1912, Body size and cell size, *J. Morphol.*, xxiii, 159.

Crozier, W. J., 1916 *a*, Cell penetration by acids, *J. Biol. Chem.*, xxiv, 255.

Crozier, W. J., 1916 *b*, Cell penetration by acids. III. Data on some additional acids, *J. Biol. Chem.*, xxvi, 225.

Crozier, W. J., 1918 *a*, Cell penetration by acids. IV. Note on the penetration of phosphoric acid, *J. Biol. Chem.*, xxxiii, 463.

Crozier, W. J., 1918 *b*, Sensory activation by acids. I. *Am. J. Physiol.*, xlv, 323.

Crozier, W. J., 1918 *c*, On indicators in animal tissues, *J. Biol. Chem.*, xxv, 455.

Haas, A. R., 1916, The permeability of living cells to acids and alkalies, *J. Biol., Chem.*, xxvii, 225.

Harvey, E. N., 1914, The permeability of cells for acids, *Internat. Z. phys.-chem. Biol.*, i, 463.

Hind, M., 1916, Studies in permeability. III, *Ann. Bot.*, xxx, 223.

Loeb, J., 1920 The proteins and colloid chemistry, *Science*, lii, 443.

Loeb, J., 1920–21 Ion series and the physical properties of proteins. I., *J. Gen. Physiol.*, iii, 85.

Osterhout, W. J. V., 1914 *a*, The effect of acid on permeability, *J. Biol. Chem.*, xix, 493.

Osterhout, W. J. V., 1914 *b*, Über den Temperaturkoeffizienten des elektrischen Leitvermögens im lebenden und toten Gewebe, *Biochem. Z.*, lxvii, 272.

Osterhout, W. J. V., 1917, Does the temperature coefficient of permeability indicate that it is chemical in nature? *Bot. Gaz.*, lxiii, 317.

Pauli, W., and Handovsky, H., 1909, Untersuchungen über physikalische Zustandsänderungen der Kolloide. VIII, *Biochem. Z.*, xviii, 340.

Stiles, W., 1920, The penetration of electrolytes into gels. I. The penetration of sodium chloride into gels of agar-agar containing silver nitrate, *Biochem. J.*, xiv, 58.

ABSORPTION OF NUTRIENTS AND PLANT GROWTH IN RELATION TO HYDROGEN ION CONCENTRATION.

By OLOF ARRHENIUS.

(From the Laboratory of Plant Physiology, Harvard University, Cambridge.)

(Received for publication, June 30, 1922.)

During the last few years studies on the effect of hydrogen ion concentration upon growth have given a clue to many hitherto unsolved problems. It would appear that this factor also plays an important rôle in the absorption of nutrient substances. Hoagland (6) in a brilliant investigation has shown that the intake of nutrients depends to a great extent on the pH value of the solution, and Hixon (4) found that the reaction of the nutrient solution affected the salt content and chemical composition of the plant.

This problem is of fundamental importance, especially in relation to the solubility of nutrient salts at different pH values. The author has therefore made some preliminary studies, the results of which are here described.

The experiments were carried out in the Laboratory of Plant Physiology of Harvard University and in the Wolcott Gibbs Memorial Laboratory. I take this opportunity to thank all who have given me assistance in the work. My warmest thanks also go to Professor W. J. V. Osterhout and Professor T. W. W. Richards for their friendly help and great kindness.

Unfortunately it was so late in the year (the experiments ran from the end of September to the end of November) that the best season of growth was passed and therefore only the results of the last month could be used. Large and rapid variations of temperature out of doors caused considerable variations in the greenhouse; some days the temperature range was 30°C., but on the average only 10°.

The experimental technique was as follows: Glass jars holding 1 gallon each were covered with black paper; in order to avoid excessive absorption of heat they were enclosed in white boards. Disks of

galvanized iron netting ($\frac{1}{4}$ inch mesh) were cut to fit inside the jars. These disks were covered with a layer of paraffin (commercial parawax). Two wires, also paraffined, were fastened on opposite sides of each disk. With a hot wire 100 holes were bored in each disk equally distant ($\frac{1}{6}$ inch) from each other. In these holes the seeds were placed. 3 liters of the nutrient solution were poured into the jar and the disk was suspended by the wire so that the solution was level with the lower surface of the disk. Then the seeds were covered with filter paper and placed in the dark for germination. After 3 days the filter paper was removed and the cultures left for 1 day in the laboratory and then transferred to the greenhouse. In order to avoid accumulation of carbon dioxide and lack of oxygen the solutions were aerated every day by pressing 2 to 3 liters of air (free from carbon dioxide) through each jar. The reaction of the solution changed considerably and it was therefore adjusted every day by adding acid or alkali to it. The solutions were changed every fortnight and the loss by transpiration measured.

During the first period of growth the germinated seeds were counted every day. After 2 weeks the cultures were thinned so that every fourth or fifth plant was left. The thinning was carried out in accordance with a definite plan, so that it was entirely a matter of chance whether a good or a bad plant was left. In this way any subjective selection was avoided. When plants are grown in any solution, those best adapted to it thrive best. Therefore it is not permissible in such an experiment as this to allow the seeds to germinate in one solution and afterward transfer them to another solution of different composition. Furthermore one should not take a given number of the best in every culture, for this would involve a selection of those best adapted and would give an erroneous result. The thinning must be done quite objectively, without taking into consideration whether the plant appears to be healthy.

When changing the solution every fortnight the length of tops and roots was measured. At the same time 1 liter of the solution was taken out for analysis. After 1 month the number of plants was reduced to 10, and after a fortnight more to 5. When the experiment was finished the plants were dried and roots and tops weighed separately.

As culture solution a Hoagland (6) solution (0.78 atmosphere) was used. This is supposed to imitate a rich soil solution. The hydrogen ion concentration was changed by adding NaOH or HCl. These were chosen because of the relatively unimportant rôle their ions play as plant nutrients.

After use as nutrient media for a fortnight the solutions were analyzed for K, Ca, Mg, PO, NO_3 and SO_4, using a new method of analysis by employing the centrifuge (1). As test plants, wheat and radish were used. In Table I certain results of the experiment are shown. The percentage of seeds germinated is found under germination per cent. Under duration of life is given the per cent of seeds living after 2 months. The rate of germination is calculated as follows: Suppose that twenty-five seeds are in the culture; after 2 days eight have germinated; after 3 days, five more; after 4 days, ten more; and between the 5th and 9th days none germinate. One divides the number of seeds germinating each day by the current number of that day and adds the results. In order to get the average this sum is divided by the total number of seeds. For the example mentioned above, one thus gets:

TABLE I.

	pH 3	4	5	6	7	8	9	10
Wheat.								
Germination, *per cent*.....	68	67	96	78	92	60	53	85
Duration of life, *per cent* ..	0	40	90	78	90	42	42	72
Rate of germination.....	0.27	0.32	0.33	0.35	0.43	0.20	0.22	0.24
Dry weight of roots, *mg*...	0	23	58	22	30	54	56	28
" " " tops, *mg*....	0	120	233	88	96	108	112	104
Radish.								
Germination, *per cent*.....	15	22	24	29	29	31	33	35
Duration of life, *per cent*...	0	0	12	15	22	15	26	21
Rate of germination.....	0.02	0.03	0.05	0.04	0.05	0.07	0.05	0.05
Dry weight of roots, *mg*...			7	10	31	18	21	7
" " " tops, *mg*. ..			38	68	87	73	113	63

$$\frac{\frac{8}{2}+\frac{5}{3}+\frac{10}{4}}{25}=0.32$$

If all seeds had germinated on the 1st day the result would be 1; if no the 2nd, 0.5; if on the 3rd, 0.33, etc. In this way the rate of germination is expressed in a more uniform and logical manner than hitherto. The table shows that the seeds germinate quite well within very wide limits; the rate of germination is more influenced by higher concentrations. This is in full agreement with the statement of Olsen (7) that the quality of germination is more dependent on the pH of the substrate than the quantity is. The determinations of dry weight give, when plotted against pH, a curve with two maxima. This seems to be a general rule for plant growth (2), as first observed by Hixon (4) and verified by later experiments. The investigations of Olsen (7) and Hoagland (5) do not speak for or against this type of curve since they have both been working on the acid side and have never gone over to the alkaline. This is because Fe, Ca, Mg and PO_4 are precipitated by increasing alkalinity. Figures on the solubility of such salts are very scarce and inaccurate, but it may be mentioned that the most insoluble calcium phosphate (the tribasic one) is soluble in about 100 to 150 parts per million (9), and as the concentration of Hoagland's solution is about 200 there will be a precipitate formed, but enough to satisfy the needs of the plant for several days will remain in solution, and as soon as part of it is used up by the plant the precipitate will be partly dissolved. It therefore seems that in this experiment the solubility effect plays a very unimportant rôle.

From Table II and Figs. 1 to 6 it is easily seen that the intake of salt is very strongly influenced by the hydrogen ion concentration. But there is no correspondence between maximal salt absorption and maximal growth of the plants as measured by weight. The curves show that, except in the case of K with radish and PO_4 and Mg with wheat, the minimum of growth corresponds approximately to the maximum absorption of salt and that in all cases, except in that of Mg with wheat and perhaps NO_3 with radish, the maxima of growth are correlated with minima, or nearly so, of the absorption curves. This, which at first glance is rather surprising, harmonizes with the fact, which has been observed by a number of investigators, that salts are absorbed in greater amounts in solutions unfavorable to growth than in favorable solutions (*cf.* Waynick (11)). The most

recent investigations on this subject are those of Osterhout (8) and Brooks (3). Brooks, investigating the permeability of *Nitella* by making spectroscopic tests of the cell sap, found that the intake of salt (in her experiments Li, Sr, and Cs were used) was greater in

TABLE II.

	Millionths of a gram-equivalent absorbed daily per plant.							
	pH 4	5	6	7	8	9	10	
Ion.								
K	4.3	2.9	4.3	0	—	1.8	0	
Ca	0	1	37	—	25	25	3	
Mg	26	30	13	0	0	4	13	
PO_4	19	4	8	21	10	8	5	
NO_3	66	10	32	29	0	4	4	Wheat.
SO_4	23	16	20	15	0	2	15	
Water, absorbed daily per plant, *cc.*	3.1	7.3	2.5	2.7	4.8	3.1	3.1	
Dry weight of roots, *mg.*	23	58	22	30	54	56	28	
" " " tops, *mg.*	120	233	88	96	108	112	104	
K		22	0	11	29	21	7	
Ca		29	1	11	23	0	0	
Mg		44	26	27	16	18	18	
PO_4		22	16	7	35	15	17	
NO_3		8	13	22	22	23	0	Radish.
SO_4		4	25	17	34	29	20	
Water absorbed daily per plant, *cc.*		1.9	3.2	3.1	4.0	3.6	2.4	
Dry weight of roots, *mg.*		7	10	31	18	21	7	
" " " tops, *mg.*		38	68	87	73	113	63	

The figures represent the average for 1 month (the last two columns for 2 weeks).

unbalanced than in balanced solutions. Similarly, we may suppose that in the solutions whose pH is most favorable for growth, the normal permeability of the plant is preserved (just as in a balanced solution) and that in consequence less salt is taken up than in less favorable solutions where the permeability has been abnormally increased. On the other hand some investigators (True and Bartlett (10)) state that the greatest absorption of salts occurs in the most

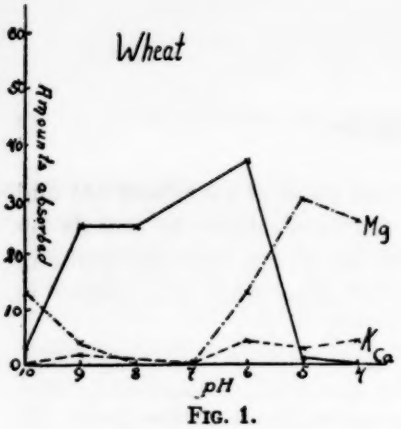

FIG. 1.

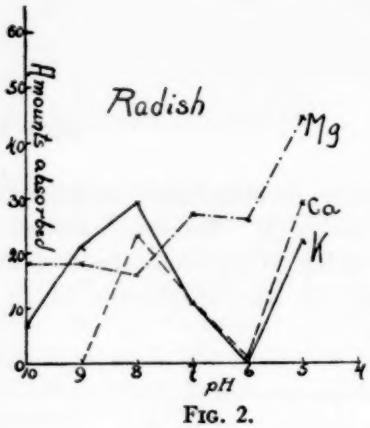

FIG. 2.

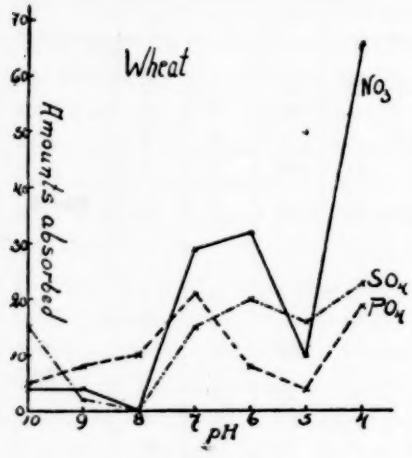

FIG. 3.

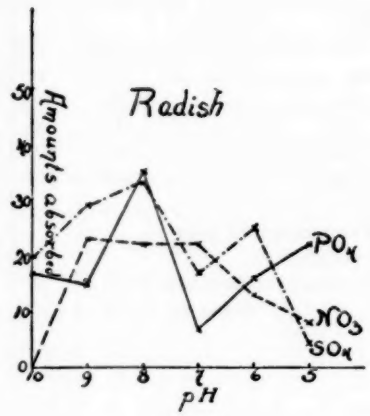

FIG. 4.

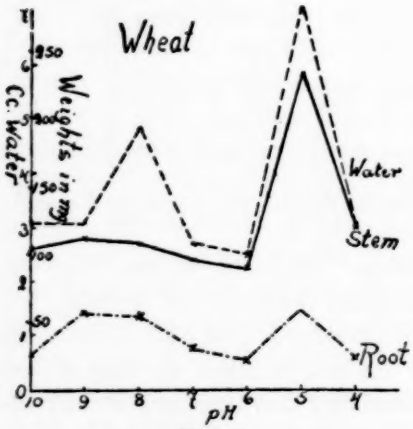

FIG. 5.

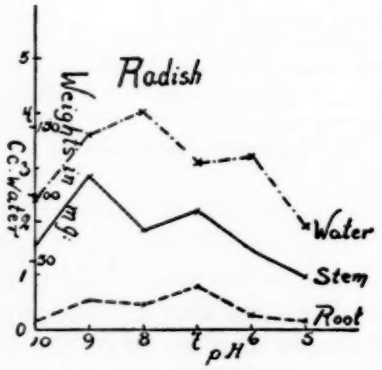

FIG. 6.

The abscissæ denote pH. The ordinates in Figs. 1 to 4 denote amounts absorbed (in millionths of a gm. equivalent); in Figs. 5 and 6 the figures at the left of the vertical line denote average daily absorption of water per plant, while those at the right denote the dry weights of stem and root.

favorable solutions. The discrepancy may be due in part to the fact that in the experiments of Waynick and the author, the concentration of nutrient substances was kept practically constant and that the pH was varied, while in the experiments of True and Bartlett this was not the case. In addition the technique and methods of measurement were entirely different.

The water is taken up quite independently of the salts, but there is a good agreement between growth and the intake of water. This is probably due in part to the increased transpiration when the leaf surface increases.

The great increase in absorption of anions by wheat in solutions of pH 4 may be explained as the result of injurious action on the roots which favors penetration, and to chemical combination, possibly due in part to the formation of protein salts, since the reaction of the solution lies on the acid side of the isoelectric point of most proteins.

Except in this case there is no selective absorption by which to explain the changes in reaction, as the result of which the solution tends to approach a point near pH 6.

SUMMARY.

The absorption of nutrients depends to a large extent on the reaction of the substrate. At maximal growth the intake of salt is at minimum. Different ions are very differently affected. The intake of water is independent of the absorption of salts.

BIBLIOGRAPHY.

1. Arrhenius, O., Quantitative analysis by centrifuge, *J. Am. Chem. Soc.*, 1922, xliv, 132.
2. Arrhenius, O., The potential acidity of soils, *Soil Sc.*, 1922.
3. Brooks, M. M., The penetration of cations into living cells, *J. Gen. Physiol.*, 1921–22, iv, 347.
4. Hixon, R. M., The effect of the reaction of a nutrient solution on germination and the first stages of plant growth, *Medd. Kungliga Vet.-Akad.*, 1920, iv, 1.
5. Hoagland, D. R., The effect of hydrogen and hydroxyl ion concentration on the growth of barley seedlings, *Soil Sc.*, 1917, iii, 547.
6. Hoagland, D. R., Relation of the concentration and reaction of the nutrient medium to the growth and absorption of the plant, *J. Agric. Research*, 1919–20, xviii, 73.

7. Olsen, C., Studier over jordbundens brintionkoncentration og dens betydning for vegetationen, soerlig for plantefordelingen i naturen, *Medd. fra Carlsberg Laboratoriet*, Copenhagen, 1921. See also Olsen, C., The concentration of hydrogen ions in the soil, *Science*, 1921, liv, 539.
8. Osterhout, W. J. V., Direct and indirect determinations of permeability, *J. Gen. Physiol.*, 1921–22, iv, 275.
9. Rindell, A., Untersuchungen über die Löslichkeit einiger Kalkphosphate, Inaugural dissertation, Helsingfors, 1898.
10. True, R. H., and Bartlett, H. H., The exchange of ions between the roots of Lupinus albus and culture solutions containing three nutrient salts, *Am. J. Bot.*, 1916, iii, 47.
11. Waynick, D. D., The chemical composition of the plant as further proof of the close relation between antagonism and cell-permeability, *Univ. California Pub. Agric. Sc.*, 1918, iii, 135.

CATAPHORETIC CHARGES OF COLLODION PARTICLES AND ANOMALOUS OSMOSIS THROUGH COLLODION MEMBRANES FREE FROM PROTEIN.

By JACQUES LOEB.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, July 7, 1922.)

I.

When an aqueous salt solution is separated from pure water (both of originally the same hydrogen ion concentration) by a collodion membrane (not treated with a protein) a diffusion of water will occur from the side of the water to the side of the solution; and this diffusion is determined not only by the difference in the osmotic pressure on the opposite sides of the membrane, but in addition also by electrical forces, due to potential differences between membrane and liquid. In these experiments the salt solution is put into a collodion bag of about 50 cc. content, closed by a rubber stopper which is perforated by a glass tube serving as a manometer, as described in previous papers.[1] If the rise of level in the manometer observed after 20 minutes at 24°C. in mm. H_2O is plotted as ordinates over the salt concentration as abscissæ, we obtain the transport curves, which differ characteristically when the substance in solution is an electrolyte or a non-electrolyte. The transport curves for solutions of non-electrolytes, like cane-sugar, resemble those for electrolytes only in higher concentrations, M/8 or above, while for lower concentrations the transport curves for non-electrolytes and certain electrolytes are typically different, since the curves for these electrolytes show a rise followed by a drop (Figs. 2, 3, and 4) both of which are lacking in non-electrolytes. The drop is followed by a second rise coinciding with the rise observed in the cane-sugar curves, and the second rise may therefore be ascribed in both cases to the purely osmotic forces of the

[1] Loeb, J., *J. Gen. Physiol.*, 1921–22, iv, 463, 621.

solution. The rise and fall of the curves at lower concentrations, however, which are only found in the case of the solutions of certain electrolytes (Figs. 2 and 3) and at definite hydrogen ion concentrations, must be ascribed to electrical forces.

In former papers the writer had arrived at the following formulation of these effects. When salt solutions are separated from pure water by collodion membranes (not treated with protein) the water is attracted by the solutions as if the water (in the pores of the membrane) were positively charged and as if it were attracted by the anion and repelled by the cation of the solution with a force which increases with the valency of the ion.

Girard,[2] and Bartell and Madison[3] had already suggested that two P.D. are active in this case, namely, first, a P.D. across the membrane between the salt solution and the water, and second, a P.D. inside the pore between the wall of the pore and the liquid diffusing through. These two P.D. are at right angles to each other. It seemed necessary to measure both P.D. at the same hydrogen ion concentrations of the solution in order to find out whether or not the transport curves can be explained on the basis of these P.D. If the P.D. across the membrane is designated as E and the P.D. inside the pore as ϵ, then, on the basis of Helmholtz's formula, the values of the electrical share of the transport curves should be parallel to the values of the product $E \times \epsilon$. It was shown in two preceding papers[1] that this was approximately true for gelatin membranes—or, more correctly, collodion membranes coated with gelatin on the solution side. These membranes behaved like gelatin membranes, the collodion acting only as a strengthening support of the gelatin film.

We intend to discuss in this paper the electrical part of the transport curves when the membranes are purely collodion membranes free from protein. The difference between the two cases is the following. When the membrane has a protein film, the charge of the water inside the pore (due to the formation of the electrical double layer) varies not only in quantity but in sign with the hydrogen ion

[2] Girard, P., *Compt. rend. Acad.*, 1908, cxlvi, 927; 1909, cxlviii, 1047, 1186; 1910, cl, 1446; 1911, cliii, 401; La pression osmotique et le méchanisme de l'osmose, Publications de la Société de Chimie-physique, Paris, 1912.

[3] Bartell, F. E., and Madison, O. E., *J. Physic. Chem.*, 1920, xxiv, 444, 593.

concentration, in a way as if the electrical double layer inside the pore were essentially determined by the Donnan equilibrium between gelatin and liquid. In the case of pure collodion membranes (free from protein), as used in the experiments for the present paper, the sign of charge of the water in the pores remains the same at any hydrogen ion concentration thus far used in the experiments. The water in the pores is practically always positively charged (except in the presence of salts with trivalent and probably tetravalent cations), while the collodion wall of the pores is negatively charged. This was ascertained by experiments on electrical endosmose[4] as well as by experiments on cataphoresis.[5]

When the water in the pores of a membrane assumes a positive charge, an electrical transport of water from the side of pure water into the solution can only take place when, in the P.D. across the membrane, the solution is negatively charged. This had already been suggested by Bartell.[3] We shall see that the results of the P.D. measurements agree with this suggestion and that they furthermore explain why the electrical transport of water into the solution increases with the valency of the anion of the salt used.

The value of ϵ, *i.e.* the P.D. between collodion and liquid, was determined by cataphoretic measurements taken from another paper in this number of this Journal.[5] The value of E, *i.e.* the P.D. across the membrane between solution and water, was measured at the beginning and the end of the diffusion experiments with the aid of a Compton electrometer and saturated KCl-calomel electrodes as described in the preceding papers. The transport curves for the same salt solutions vary with the hydrogen ion concentration. At the beginning of the experiment salt solutions and water were brought to the same pH, the pH being determined colorimetrically. When acid was required HCl was added, and when alkali was required KOH was used.

Fig. 1 gives the transport curves of water from the side of water into salt solutions at an initial pH of 3.0, the acid used being in all cases HCl. The ordinates of the curves are the rise of liquid in the manometers (connected with the solutions) after 20 minutes; the abscissæ

[4] Loeb, J., *J. Gen. Physiol.*, 1919–20, ii, 563.
[5] Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 109.

are the initial concentrations of the solutions. The curves in Fig. 1 are similar to the curves obtained with non-electrolytes like cane-sugar, and hence are entirely or chiefly due to the transport of water by purely osmotic forces. There is no indication of any transport due to electric forces.

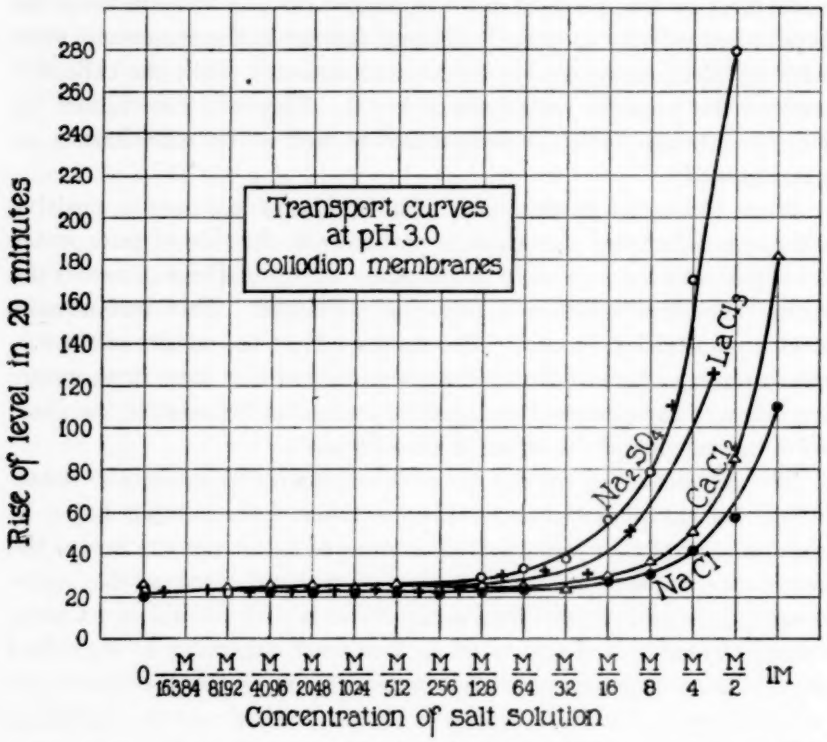

FIG. 1. Transport curves at pH 3.0 through collodion membranes. The ordinates are the rise of level in mm. H_2O in the manometers connected with the solution; the abscissæ are the concentrations of salt solutions. The curves show that at this pH the diffusion of water is determined purely by osmotic and not by electrical forces.

This result finds its explanation in the values given in Tables I, II, and III. In Tables I and II are given the P.D. across the membrane (E) at the beginning of the experiments and at the end (*i.e.*, after 20 minutes). The method of these measurements was described in a previous article. The solution was always positively charged with

TABLE I.

P.D. in millivolts between salt solutions and H_2O, of pH 3.0, across collodion membranes, *at beginning of experiment*. The minus or plus signs designate the sign of charge on the solution side of the membranes.

Concentration.....	0	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2	1 M	
Na_2SO_4.....	0	−1.0	+1.0	−1.0	−1.0	−1.5	− 2.5	− 3.5	− 5.0	− 6.5	− 9.0	−12.0	−16.0	−20.0		
KCl.....	+1.5		+0.5	0	+0.5	+0.5	+ 0.5	+ 0.5	+ 1.0	+ 1.0	+ 0.5	+ 1.5	+ 1.0	+ 1.5	+ 1.5	
NaCl.....	+1.0		+0.5	+1.5	+2.0	+3.0	+ 4.5	+ 7.0	+ 9.0	+11.0	+13.5	+16.0	+18.0	+21.0	+22.5	
LiCl.....	+1.0		+3.0	+3.0	+3.0	+5.0	+ 7.0	+10.0	+13.0	+17.5	+23.0	+27.0	+31.0	+34.0	+37.0	
$CaCl_2$.....	+2.0	+2.5	+4.0	+6.0	+7.0	+9.0	+13.0	+17.0	+22.0	+27.0	+31.0	+36.0	+39.0	+41.0	+48.0	
Concentration.....	0	M/49,152	M/24,576	M/12,288	M/6,144	M/3,072	M/1,536	M/768	M/384	M/192	M/96	M/48	M/24	M/12	M/6	M/3
$LaCl_3$.....	+2.0	+1.5	+2.0	+2.5	+3.0	+5.0	+ 6.0	+ 9.0	+13.5	+18.0	+23.0	+29.0	+34.0	+41.5	+48.0	+53.0

TABLE II.

P.D. in millivolts between salt solutions and H_2O, of pH 3.0, across collodion membranes, *at end of experiment*. The minus or plus signs designate the sign of charge on the solution side of the membranes.

Concentration.....	0	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2	1 M	
Na_2SO_4.....	+1.0	0	0	−1.5	−2.0	−3.5	− 5.0	− 6.0	− 6.0	− 6.5	− 7.0	−10.5	−11.0	− 9.0		
KCl.....	+0.5		+0.5	+0.5	0	0	+ 0.5	0	− 0.5	0	0	0	− 0.5	− 0.5	0	
NaCl.....	+1.0		+1.5	+1.0	+1.5	+2.0	+ 3.5	+ 6.5	+ 7.5	+ 9.0	+10.0	+11.0	+10.0		+ 7.0	
LiCl.....	+0.5		+1.0	+2.5	+3.0	+4.0	+ 6.5	+ 8.0	+12.5	+16.0	+19.0	+19.0	+18.0	+17.5	+14.5	
$CaCl_2$.....	+2.0	+3.0	+4.0	+5.0	+7.5	+9.0	+12.5	+16.5	+19.0	+22.0	+22.5	+22.5	+22.0	+21.0	+18.5	
Concentration.....	0	M/49,152	M/24,576	M/12,288	M/6,144	M/3,072	M/1,536	M/768	M/384	M/192	M/96	M/48	M/24	M/12	M/6	M/3
$LaCl_3$.....	+1.5	+2.0	+2.0	+2.0	+2.5	+3.0	+ 5.0	+ 9.0	+13.0	+18.0	+21.0	+23.5	+27.0	+25.0	+24.0	+25.0

the exception of the Na_2SO_4 solution; but at low concentrations of Na_2SO_4 the value of E was too small to have any effect, while at higher concentrations the value of ϵ became rather small. In Table III are given the cataphoretic P.D. (ϵ) between collodion particles and water in the same solutions as those mentioned in Tables I and II.

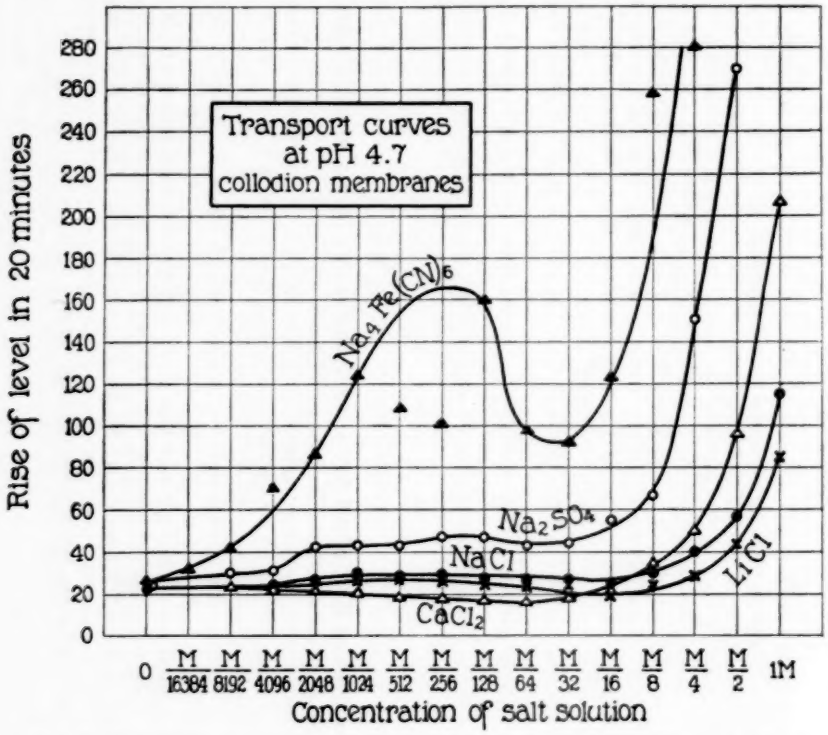

FIG. 2. Transport curves at pH 4.7. The transport curves for $Na_4Fe(CN)_6$ and Na_2SO_4 show a slight electrical effect in concentrations below M/32. The transport curves for NaCl, LiCl, and $CaCl_2$ show no electrical effect.

The method of the measurements of the cataphoretic P.D. was that described by Northrop.[6] As Table III shows, the sign of charge of the liquid in the pores of the membrane is also positive. Since the solution and the water inside the pores always have the same sign of charge (except in the case of Na_2SO_4 mentioned above), no

[6] Northrop, J. H., *J. Gen. Physiol.*, 1921–22, iv, 629.

TABLE III.

Cataphoretic P.D. in millivolts between collodion particles and aqueous solutions at pH 3.0. The plus sign designates the charge of water.

Concentration	0	M/32,768	M/16,384	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4
Na_2SO_4	+63			+72	+72	+63	+73	+68	+65	+51	+46	+35	+28		+12
NaCl	+60			+62	+60	+57	+59	+59		+46	+39	+38	+29	+20	+14
$CaCl_2$	+62			+40	+37	+35	+34	+31	+27	+24	+19	+16	+14	+10	+ 7
$LaCl_3$	+68	+32	+28	+22	+18	+14	+ 8	+ 7	+ 5	+ 3	+ 1	0	0		

TABLE IV.

P.D. in millivolts between salt solutions and H_2O, of pH 4.7, across collodion membranes, *at beginning of experiment.* The minus or plus signs designate the sign of charge of the solutions.

Concentration	0	M/16,384	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2	1 M
$Na_4Fe(CN)_6$	+1.0	−8.0	−15.0	−21.0	−32.0	−29.0	−28.0	−27.5	−31.5	−32.5	−31.0	−40.0	−46.0	−52.5		
Na_2SO_4	+1.5		− 5.5	−10.0	−13.5	−15.0	−14.0	−14.0	−15.0	−17.5	−20.0	−22.0	−24.0	−27.5	−30.0	
KCl	−1.0			− 1.5	− 3.0	− 4.0	− 4.0	− 4.0	− 3.0	− 1.5	− 2.0	− 1.5	− 1.5	− 1.0	0	0
NaCl	+1.5			+ 4.5	+ 2.5	+ 6.5	+10.0	+11.0	+17.5	+21.0	+20.0	+27.0	+28.0	+31.0	+35.0	+35.0
LiCl	0			+ 4.0	+ 6.0	+ 9.5	+14.0	+19.0	+25.0	+33.0	+38.0	+45.0	+48.0	+54.0	+51.0	+59.0
$CaCl_2$	+2.5		+ 6.5	+ 9.0	+14.5	+21.0	+25.0	+31.5	+37.5	+43.5	+47.0	+52.0	+59.0	+61.5	+67.0	+67.0

TABLE V.

P.D. in millivolts between salt solutions and H_2O, of pH 4.7, across collodion membranes, *at end of experiment*. The minus or plus signs designate the sign of charge of the solutions.

Concentration.....	0	M/16,384	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2	1 M
$Na_4Fe(CN)_6$.....	−2.0	−12.5	−17.5	−25.0	−34.0	−34.0	−25.0	−24.0	−26.0	−21.5	−20.0	−18.5	−21.0	−25.0		
Na_2SO_4.....	−4.0		− 7.5	−14.0	−15.0	−13.5	−17.0	−12.5	−12.5	−11.5	−11.0	−11.5	−11.0	−11.5	−12.5	
KCl.....	−8.0			− 7.0	− 8.0	− 7.5	− 8.0	− 4.0	− 3.0	− 3.0	− 1.5	− 0.5	0	0	0	0
NaCl.....	−1.0			− 1.5	− 2.0	+ 1.5	+ 5.0	+ 4.5	+ 9.0	+10.0	+ 9.5	+10.0	+ 9.0	+10.0	+ 8.5	+ 7.5
LiCl.....	−4.0			− 2.5	− 2.5	− 1.5	+ 7.5	+14.0	+18.5	+19.0	+21.0	+20.5	+20.0	+19.0	+17.0	+17.0
$CaCl_2$.....	−1.0		+ 2.5	+ 4.0	+10.0	+ 9.0	+17.5	+22.0	+24.5	+24.0	+23.5	+24.0	+23.5	+20.5	+20.5	+20.5

TABLE VI.

Cataphoretic P.D. in millivolts between collodion particles and aqueous solutions at pH 4.7. The plus sign designates the charge of water.

Concentration.....	0	M/32,768	M/16,384	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2
$Na_4Fe(CN)_6$.....	+29	+51	+55	+60	+64	+68	+65	+57	+50	+42	+30	+21	+13	+ 9		
Na_2SO_4.....	+28	+47	+47	+56	+59	+65	+70	+69	+63	+56	+43	+36	+26	+19	+13	
KCl.....	+32	+31	+37	+45	+52	+53	+62	+66	+66	+60	+49	+40	+30	+21	+14	
NaCl.....	+26			+47	+53	+58	+60	+64	+61	+55	+49	+37	+30	+20	+14	+10
LiCl.....	+31	+41	+42	+48	+47	+57	+60	+64	+68	+61	+55	+44	+36	+22	+17	
$CaCl_2$.....	+36		+30	+31	+32	+35	+32	+28	+26	+22	+17	+14	+10	+ 8	+ 6	
$LaCl_3$.....	+29	+17	+19	+19	+21	+14	+10	+ 8	+ 4	+ 4	0	0	− 3	− 6		

electrical transport of water into the solution should be possible, as was found to be the case.

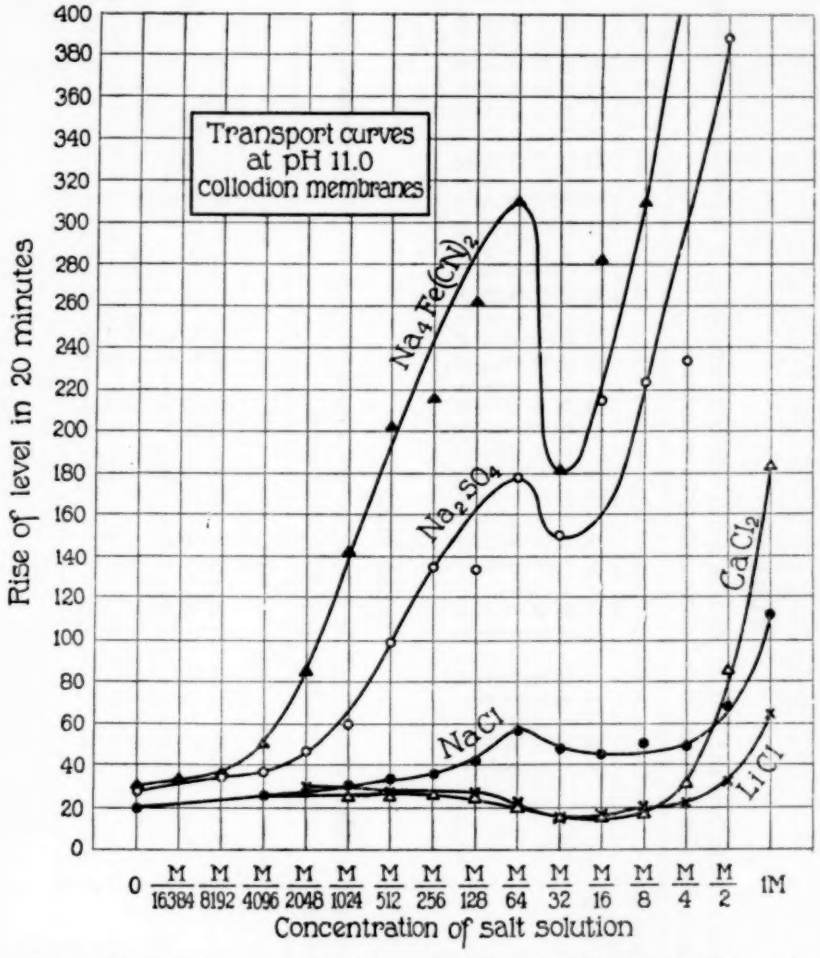

FIG. 3. Transport curves at pH 11.0. The electrical effect is visible only for NaCl, Na_2SO_4, and $Na_4Fe(CN)_6$ for concentrations below M/64.

Fig. 2 gives the transport curves at a lower hydrogen ion concentration; namely, pH 4.7. We now notice a marked electrical effect in the transport curves for $Na_4Fe(CN)_6$ and a slight one for Na_2SO_4. This is accounted for in Tables IV, V and VI. Tables IV and V show that

TABLE VII.

P.D. in millivolts between salt solutions and H_2O, of pH 11.0, across collodion membranes, *at beginning of experiment.* The minus or plus signs designate the sign of charge on the solution side of the membranes.

Concentration.....	0	M/16,384	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2	1 M
$Na_4Fe(CN)_6$.....	−1.5	−2.5	−5.0	−8.5	−13.0	−19.0	−22.5	−30.0	−27.0	−30.5	−29.0	−37.5	−43.0	−47.5		
Na_2SO_4.....	−2.0		−4.5	−6.0	− 8.0	−14.0	−17.0	−18.0	−17.0	−18.0	−17.0	−19.0	−21.0	−24.0	−29.0	
KCl.....	0			−2.5	− 3.0	− 3.0	− 5.0	− 4.0	− 4.0	− 3.5	− 2.5	− 2.5	− 1.5	− 1.5	− 1.0	− 1.0
NaCl.....	0			−1.5	− 2.0	− 2.5	− 1.5	− 1.5	+ 1.0	+ 2.0	+ 6.0	+10.0	+13.0	+16.0	+19.0	+22.0
LiCl.....	−0.5			−1.5	− 2.5	− 1.5	0	+ 3.5	+ 7.5	+13.0	+18.0	+23.0	+27.0	+32.5	+38.0	+41.0
$CaCl_2$.....	−2.5		−2.5	−2.0	− 1.0	+ 1.0	+ 5.0	+10.0	+15.0	+20.0	+26.0	+31.5	+37.0	+42.0	+46.5	+51.0

TABLE VIII.

P.D. in millivolts between salt solutions and H_2O, of pH 11.0, across collodion membranes, *at end of experiment.* The minus or plus signs designate the sign of charge on the solution side of the membranes.

Concentration.....	0	M/16,384	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2	1 M
$Na_4Fe(CN)_6$.....	−3.0	−3.5	−5.0	−7.5	−12.0	−20.0	−25.0	−24.0	−26.0	−25.0	−21.0	−22.5	−23.0	−24.0		
Na_2SO_4.....	−2.5		−4.5	−6.5	− 6.5	−13.5	−16.0	−18.0	−16.0	−16.0	−14.0	−15.0	−14.0	−13.0	−19.0	
KCl.....	−2.0			−2.0	− 2.5	− 3.0	− 4.0	− 4.0	− 3.5	− 3.0	− 2.5	− 2.0	− 1.0	− 0.5	0	0
NaCl.....	−0.5			− 2.0	− 2.0	− 3.0	− 2.5	− 1.5	− 0.5	+ 1.5	+ 1.5	+ 6.0	+ 7.0	+ 7.5	+ 7.5	+ 8.0
LiCl.....	−2.5			− 3.5	− 2.5	− 2.5	− 1.0	+ 3.0	+ 7.5	+10.0	+13.0	+17.0	+18.0	+16.5	+13.0	+15.0
$CaCl_2$.....	−2.0		−2.5	−3.0	− 1.5	− 1.0	+ 5.0	+ 8.5	+13.5	+16.5	+19.5	+22.0	+21.0	+23.0	+20.0	+18.0

TABLE IX.

Cataphoretic P.D. in millivolts between collodion particles and aqueous solutions at pH 11.0. The plus sign designates the sign of charge of the water.

Concentration	0	M/32,768	M/16,384	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4
$Na_4Fe(CN)_6$	+59	+64	+66	+74	+71	+71	+70	+69	+52	+41	+32	+24	+16		
Na_2SO_4	+60	+69	+70	+70	+71	+73	+75	+65	+63	+56	+49	+35	+28	+20	
NaCl	+62		+62	+66	+65	+66	+65	+66	+65	+62	+53	+43	+34	+24	+18
$CaCl_2$	+58	+48	+48	+40	+39	+37	+34	+29	+28	+24	+19	+15	+ 9	+ 6	

the solutions of $Na_4Fe(CN)_6$ and Na_2SO_4 assume a considerable negative charge in the P.D. across the membrane and that this P.D. is greater in the case of $Na_4Fe(CN)_6$ than in the case of Na_2SO_4. Since, according to Table VI, the water in the pores is positively charged, an electrical transport must occur. In KCl there is a slight negative charge of the solution (Table IV) but too small to have any effect on the transport of water. In the case of NaCl, LiCl, $CaCl_2$, and $LaCl_3$ no electrical transport is possible since the solution as well as the water in the pores are positively charged.

Observations taken at pH 5.8 were similar to those at pH 4.7 except that the effects of Na_2SO_4 and $Na_4Fe(CN)_6$ were greater than at pH 4.7. The P.D. across the membrane was also greater for these salts. Since otherwise everything was similar to the result at pH 4.7, further discussion of the results at pH 5.8 may be omitted.

Fig. 3 gives the transport curves at pH 11.0, the solutions being brought to this pH by the addition of KOH. $Na_4Fe(CN)_6$, Na_2SO_4, NaCl, and KCl (not shown in the figure) gave electrical transport curves. In this case the salt solutions mentioned were negatively charged (Tables VII and VIII), while the water in the pores was as usual positively charged (Table IX); the negative charge in the solution (Tables VII and VIII) increased in the order of KCl and NaCl $<$ Na_2SO_4 $<$ $Na_4Fe(CN)_6$, as did the electrical part of the transport curves in Fig. 3. In the case of LiCl and $CaCl_2$ the solutions were positively charged and hence the transport curves are the effect of the osmotic forces alone.

The P.D. measurements therefore explain the transport curves in a semiquantitative way in all these experiments.

II.

While all these results show that the electrical transport of water in these experiments varies semiquantitatively with the value of the product $E \times \epsilon$, some difficulties are encountered when we compare the effect of Na_2SO_4 or $Na_4Fe(CN)_6$ on the electrical transport at pH 11.0 with that at pH 5.8. In Fig. 4 are presented the transport curves for Na_2SO_4 at different pH, varying from 2.0 to pH 12.0.

At pH 2.0 and pH 3.0 the P.D. across the membrane (Table X) is small, and this accounts for the fact that at both pH the transport

curves are purely osmotic in character (Fig. 4). At pH 4.7 the electrical transport of liquid from water into the Na_2SO_4 solution

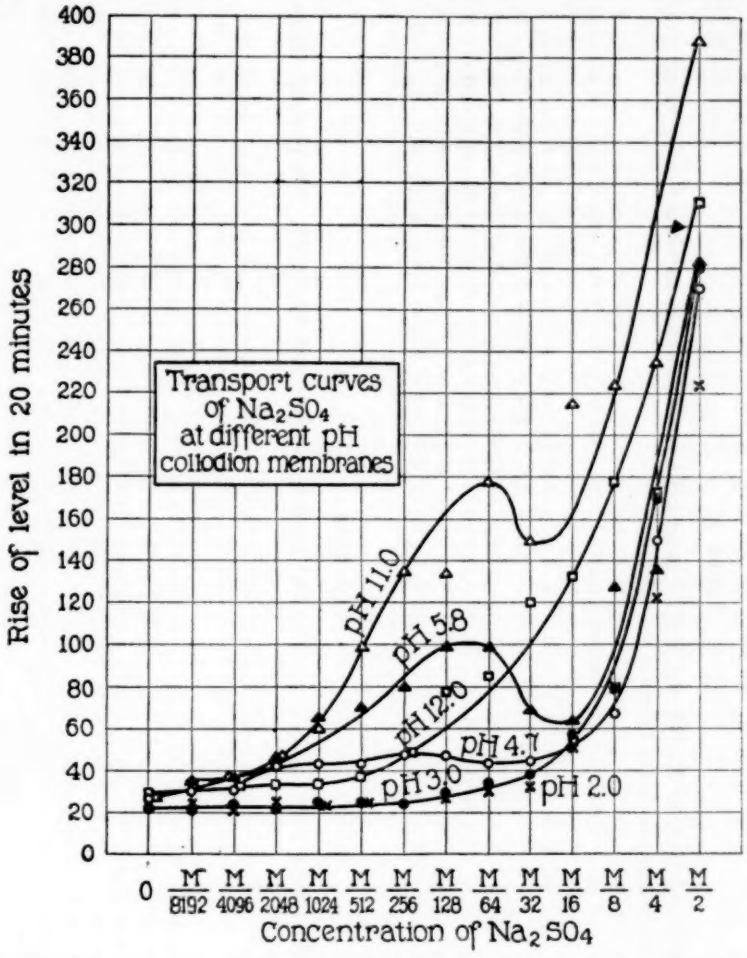

FIG. 4. Transport curves for Na_2SO_4 at different pH showing that the electrical effect for the same salt varies considerably with the hydrogen ion concentration of the solution.

becomes noticeable, as already mentioned. At pH. 5.8 (Fig. 4) the electrical transport of water from the side of pure water into the Na_2SO_4 solution is greater than at pH 4.7, and at pH 11.0

TABLE X.

P.D. in millivolts between solutions of Na_2SO_4 and H_2O of same pH across a collodion membrane, *at beginning of experiment.* The plus or minus sign designates the sign of charge of the solutions.

Concentration of Na_2SO_4	0	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2
pH 2.0	+0.5	+ 0.5	+ 0.5	+ 0.5	+ 1.0	+ 1.0	+1 .0	+ 1.0	+ 1.0	0	− 1.0	− 4.0	− 7.0	−12.0
pH 3.0	0	− 1.0	+ 1.0	− 1.0	− 1.0	− 1.5	− 2.5	− 3.5	− 5.0	− 6.5	− 9.0	−12.0	−16.0	−20.0
pH 4.7	+1.5	− 5.5	−10.0	−13.5	−15.0	−14.0	−14.0	−15.0	−17.5	−20.0	−22.0	−24.0	−27.5	−30.0
pH 5.8		−20.0	−21.0	−30.0	−29.0	−23.0	−22.0	−24.0	−21.0	−15.0	−24.0	−30.0	−26.0	−34.0
pH 11.0	−2.0	− 4.5	− 6.0	− 8.0	−14.0	−17.0	−18.0	−17.0	−18.0	−17.0	−19.0	−21.0	−24.0	−29.0
pH 12.0	0	− 0.5	− 1.0	− 1.0	− 1.0	− 1.5	− 3.0	− 4.0	− 5.5	− 8.0	−10.5	−13.5	−16.5	−21.0

TABLE XI.

Cataphoretic P.D. in millivolts between collodion particles and Na_2SO_4 solutions at different pH. The plus sign designates the sign of charge of the water.

Concentration of Na_2SO_4	0	M/32,768	M/16,384	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4
pH 2.0	+37	+43	+43	+54	+41	+40	+42	+41	+42	+38	+32	+25	+19	+10	
pH 3.0	+63			+72	+72	+63	+73	+68	+65	+51	+46	+35	+28	(+6)	+12
pH 4.7	+28	+47	+47	+56	+59	+65	+70	+69	+63	+56	+43	+36	+26	+19	+13
pH 5.8	+21	+50	+58	+53	+60	+64	(+90)	+67	+62	+55	+46	+36	+27	+21	
pH 11.0	+60	+69	+70	+70	+71	+73	+75	+65	+63	+56	+49	+35	+28	+20	
pH 12.0	+45	+47	+50	+50	+49	+52	+50	+49	+47	+42	+37	+31	+25	+19	

(Fig. 4) the electrical transport of water is still greater than at pH 5.8. At pH 12.0 (Fig. 4) the electrical transport diminishes again. The fact that the electrical transport is less at pH 12.0 than at pH 11.0 finds its explanation in Table X showing that the P.D. across the membrane is considerably smaller at pH 12.0 than at pH 11.0.

A difficulty arises, however, if we wish to account for the fact that the electrical transport curve for Na_2SO_4 is higher at pH 11.0 than that at pH 5.8, since the P.D. across the membrane is greater at pH 5.8 than at pH 11.0 (Table X), while Table XI shows that the value of ϵ is practically identical at both pH.

The same difficulty occurs in the case of the transport curves for $Na_4Fe(CN)_6$ at pH 5.8 and 11.0, these transport curves being higher at pH 11.0 than at pH 5.8, although the values $E \times \epsilon$ do not warrant the difference in the transport curves. It seems to follow that some other variable in the equation for the diffusion of water (besides E and ϵ) is affected by the pH. The low value of the electrical transport in Na_2SO_4 solutions at pH 2.0 is accounted for by the low value of the P.D. across the membrane (Table X).

The electrical part of the transport curves lies in a concentration of the salts between zero and about M/8 or M/4. The transport curves for $Na_4Fe(CN)_6$ or Na_2SO_4 rise at first with increasing concentration until a maximum is reached at a molecular concentration of between M/32 or M/16, then drop slightly, and then rise again sharply (Figs. 2, 3, and 4). This latter rise is probably due exclusively to the osmotic effect of the salt in solution and need not be considered for our present purpose. The initial rise in the curves with increasing concentration of Na_2SO_4 or $Na_4Fe(CN)_6$ is, however, electrical in character and is accounted for by a corresponding rise in the value of E with increasing concentration, as is shown in Tables IV, VII, and X.

The drop of the electrical transport curves at a concentration of between M/32 and M/16 at pH 5.8 and pH 11.0 is explained by a corresponding drop in values of ϵ as is shown in Tables VI and IX. We can therefore say that from the influence of electrolytes on the P.D. across the membrane (E) and on the cataphoretic P.D. (ϵ) it is possible to explain semiquantitatively the phenomena of anomalous osmosis through a collodion membrane with the exception of some minor discrepancies.

III.

We may finally inquire into the nature of the P.D. across the membrane. It was pointed out that it was essentially, but perhaps not exclusively, a diffusion potential due to the difference in the rate of migration of the oppositely charged ions of a salt through the membrane.

The liquid in the pores of a collodion membrane is generally positively charged; only solutions of salts with trivalent (and probably tetravalent) cations can, in sufficiently high concentrations, cause the liquid to be negatively charged. If we omit these exceptions, it follows that an electrical transport of liquid through the membrane from the side of the water into the solution can only occur when in the P.D. across the membrane a sufficiently large negative charge is found on the solution side. The experiments show that this occurs in a pronounced way only when the anion of the salt is plurivalent, *i.e.* SO_4, oxalate, PO_4, $Fe(CN)_6$, etc., while the cation is univalent, and the question arises, why this should be so. The answer is given by the measurements of diffusion potentials of solutions of salts against pure water of the same pH without the interposition of a membrane, which show that in the diffusion potentials of solutions of salts with plurivalent anions and univalent cations against water, the solutions assume a negative charge which increases with the valency of the anions and the concentration of the salts. This is obvious from the results in Table XII giving the diffusion potentials for a series of salt solutions against water without the interposition of a membrane at pH 4.7. When the anion is plurivalent and the cation univalent, the solution assumes a negative charge in the P.D. across the membrane and the negative charge increases with the valency of the anion and the concentration of the salt. The same is true for the charge of the solution when the diffusion occurs through a collodion membrane. Since the salts can diffuse through the collodion membrane, diffusion potentials are bound to occur and we cannot doubt that these diffusion potentials explain the peculiar effect which the sign of charge and valency of ion have on the electrical part of the transport curves; *i.e.*, on anomalous osmosis. The P.D. across the membrane is essentially due to the diffusion potential of the solution.

TABLE XII.

Diffusion potentials in millivolts between salt solutions and H_2O of pH 4.7.

Concentration	M/16,384	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4	M/2	1 M
$Na_4Fe(CN)_6$	−2.5	− 4.0	− 7.0	−10.0	−14.0	−18.0	−24.0	−27.0	−32.0	−36.5	−43.0	−49.0	−55.0		
Na_2SO_4		− 2.5	− 4.0	− 5.5	− 7.0	− 9.0	−11.5	−13.5	−15.5	−18.0	−23.0	−26.0	−29.0	−34.0	
KCl		0	+ 1.0	+ 0.5	+ 0.5	+ 0.5	+ 0.5	0	0	0	0	0	0	0	0
NaCl	0	+ 7.0	+ 9.0	+11.5	+14.0	+18.0	+20.5	+22.5	+25.0	+27.0	+29.5	+33.5	+36.0	+39.0	+42.0
LiCl	0	+ 6.0	+ 9.5	+16.5	+20.5	+23.5	+30.0	+37.5	+42.5	+46.5	+50.0	+55.0	+60.0	+63.5	+68.5
$CaCl_2$		+16.0	+21.0	+26.5	+31.0	+35.0	+41.0	+47.0	+53.0	+59.0	+64.5	+70.0	+76.0	+78.0	+84.0
$BaCl_2$	+ 6.5	+10.0	+15.0	+20.0	+26.0	+31.5	+36.5	+43.0	+47.5	+52.5	+57.0	+61.5	+67.5	+75.0	
$MgCl_2$	+ 7.0	+11.0	+17.5	+24.0	+30.0	+36.0	+41.0	+46.5	+53.5	+62.0	+68.0	+72.5	+78.5	+86.5	+96.5
$MgSO_4$	+ 5.0	+ 6.0	+ 6.0	+ 7.5	+ 7.5	+ 8.5	+ 9.0	+10.0	+11.0	+11.0	+11.0	+11.5	+11.0	+10.0	
$LaCl_3$	+10.0	+14.0	+19.0	+24.0	+29.0	+36.0	+43.0	+49.0	+55.0	+61.0	+67.0	+74.0	+80.0	+88.0	

TABLE XIII.

P.D. in millivolts across the membrane minus diffusion potential without membrane, at pH 4.7.

Concentration	M/8,192	M/4,096	M/2,048	M/1,024	M/512	M/256	M/128	M/64	M/32	M/16	M/8	M/4
$Na_4Fe(CN)_6$	−11.0	−14.0	−22.0	−15.0	−10.0	−3.5	−4.5	− 0.5	+5.5	+ 3.0	+ 3.0	+ 2.5
Na_2SO_4	− 3.0	− 6.0	− 8.0	− 8.0	− 5.0	−2.5	−1.5	− 2.0	−2.0	+ 1.0	+ 2.0	+ 1.5
NaCl		− 4.5	− 9.0	− 7.5	− 8.0	−9.5	−5.0	− 4.0	−7.0	− 2.5	− 5.5	− 5.0
$CaCl_2$	− 9.5	−12.0	−12.0	−10.0	−10.0	−9.5	−9.5	− 9.5	−12.0	−12.5	−11.0	−14.5

While the diffusion potentials of salt solutions against water without a membrane agree to a large extent with the P.D. across the collodion membrane, the two P.D. are by no means identical. If the diffusion potentials without interposition of a membrane in Table XII are compared with the P.D. across the collodion membrane at pH 4.7 given in Table IV, it is noticeable that the values for diffusion potentials differ in a definite sense from the values for the P.D. across the membrane, especially for the concentrations below M/128 or M/256. This difference between P.D. across the membrane minus diffusion potential without a membrane has in this case always a negative value, as is shown in Table XIII. It looks as if the rate of diffusion of the anions was comparatively more retarded by the membrane than the rate of diffusion of the cations. Since the collodion membrane is negatively charged, it might be argued that the retardation is due to a repulsion of the anions by the negatively charged membrane and an attraction of the positive ion of the salt. While this is possible, it is also possible that another P.D. is superposed upon the diffusion potential. It will be necessary to return to this subject in a subsequent paper.

SUMMARY AND CONCLUSIONS.

1. It had been shown in previous papers that when a salt solution is separated from pure water by a collodion membrane, water diffuses through the membrane as if it were positively charged and as if it were attracted by the anion of the salt in solution and repelled by the cation with a force increasing with the valency. In this paper, measurements of the P.D. across the membrane (E) are given, showing that when an electrical effect is added to the purely osmotic effect of the salt solution in the transport of water from the side of pure water to the solution, the latter possesses a considerable negative charge which increases with increasing valency of the anion of the salt and diminishes with increasing valency of the cation. It is also shown that a similar valency effect exists in the diffusion potentials between salt solutions and pure water without the interposition of a membrane.

2. This makes it probable that the driving force for the electrical transport of water from the side of pure water into solution is primarily a diffusion potential.

3. It is shown that the hydrogen ion concentration of the solution affects the transport curves and the diffusion potentials in a similar way.

4. It is shown, however, that the diffusion potential without interposition of the membrane differs in a definite sense from the P.D. across the membrane and that therefore the P.D. across the membrane (E) is a modified diffusion potential.

5. Measurements of the P.D. between collodion particles and aqueous solutions (ϵ) were made by the method of cataphoresis, which prove that water in contact with collodion particles free from protein practically always assumes a positive charge (except in the presence of salts with trivalent and probably tetravalent cations of a sufficiently high concentration).

6. It is shown that an electrical transport of water from the side of water into the solution is always superposed upon the osmotic transport when the sign of charge of the solution in the potential across the membrane (E) is opposite to that of the water in the P.D. between collodion particle and water (ϵ); supporting the theoretical deductions made by Bartell.

7. It is shown that the product of the P.D. across the membrane (E) into the cataphoretic P.D. between collodion particles and aqueous solution (ϵ) accounts in general semiquantitatively for that part of the transport of water into the solution which is due to the electrical forces responsible for anomalous osmosis.

THE INFLUENCE OF ELECTROLYTES ON THE CATAPHORETIC CHARGE OF COLLOIDAL PARTICLES AND THE STABILITY OF THEIR SUSPENSIONS.

I. Experiments with Collodion Particles.

By JACQUES LOEB.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, July 26, 1922.)

I.

INTRODUCTION.

The work of McTaggart[1] on the electrification of water-gas surfaces is of fundamental importance for the question concerning the origin of the potential differences responsible for the cataphoresis of solid particles in aqueous solutions. McTaggart found that the P.D. between gas bubbles and water is about 55 millivolts, the water assuming a positive and the bubble a negative charge. The nature of the gas had apparently no influence on the P.D. and even particles of ice moving in water had the same P.D. From this he concludes that these cataphoretic P.D. are determined exclusively by the water. When the bubble moves, one surface of the double layer is fixed to the gas bubble and "preserves its identity more or less as it moves through the liquid." This layer must have an excess of OH ions when the bubble assumes its usual negative charge. He also points out that "the fact that so many substances in contact with water are negatively charged indicates that water by itself produces an electrification upon which is superposed an electrification due to the substance in contact with it."

On the basis of experiments on waterfall electricity Lenard[2] also arrived at the conclusion that the cause for the formation of an

[1] McTaggart, H. A., *Phil. Mag.*, 1914, xxvii, 297; xxviii, 367.

[2] Lenard, P., *Ann. Physik*, 1915, xlvii, 463.

electrical double layer at water surfaces must be in the water itself, and that both strata of the double layer between a colloidal particle and water must lie entirely in the water. Waterfall electricity is due to the mechanical tearing off of very minute particles of water from the surface. Lenard observed that when the particles torn off were very minute they were negatively charged, while when they were larger they were electrically neutral. According to McTaggart it is, however, not entirely certain that the double layer which surrounds a bubble of air in water and is active in a cataphoresis experiment is the same as that which is responsible for waterfall electricity.

The question arises: What causes this ionic stratification near the surface of the liquid? Since salts raise the surface tension of water, they must be contained in the surface in lower concentration than in the body of liquid. This was already emphasized by McTaggart. Should it be possible that the OH and H ions have a different effect on the surface tension, the H ions causing a greater rise of the surface tension of water than the OH ions? In that case the concentration of the OH ions should be higher in the outermost, *i.e.*, most superficial, stratum of the surface of the water than the concentration of the H ions, while the concentration of H ions should be greater in the layer of water underneath. This would explain why water generally assumes a positive charge in contact with colloidal particles, provided that the most superficial layer of water adheres to the particle moving in the electric field. It is, however, not necessary to make any definite assumption in regard to the nature of the forces responsible for the ionic stratification beyond stating that they must be "molecular forces" *inherent in the water itself*.

There are, however, cases where the cataphoretic P.D. seems to have a different origin from that of air bubbles moving in an electric field. This is apparently true for protein particles where the charge is obviously connected with the ionization of the particles. Thus it is well known through the experiments of Hardy, Michaelis,[3] and others that such particles do not migrate in an electric field at the hydrogen ion concentration of their isoelectric point, while they migrate towards the cathode when the hydrogen ion concentration is higher and

[3] Michaelis, L., Die Wasserstoffionenkonzentration, 2nd edition, Berlin, 1922.

towards the anode when the hydrogen ion concentration is lower than that of the isoelectric point of the protein. It is obvious that the ionization of these particles controls their cataphoretic P.D.

It seemed of interest to compare the influence of neutral salts on these two types of cataphoretic potentials. A convenient material was obtained in the form of fine collodion particles which were found to behave cataphoretically very much like air bubbles. When such collodion particles were coated with a protein, *e.g.*, gelatin, they could be used for the study of the cataphoretic potentials of the protein with which the particles were coated. In this paper we shall report only on the experiments with pure collodion particles *free from contamination with protein.*

The method of the evaluation of the cataphoretic P.D. between small particles and aqueous solutions was based on mobility measurements of individual particles by cataphoresis under the microscope, in which the apparatus with non-polarizable electrodes decribed by Northrop[4] was used. The apparent depth of the cell for the mobility measurements was 0.6 mm. and the time required for a particle to travel a distance of 45μ or when necessary 90μ was measured for four depths; namely, 0, 0.1, 0.2, and 0.3 mm., to eliminate the influence of the cataphoresis of the water itself. These measurements were averaged and the mobility in centimeters per second per volt per centimeter $\times$ 10^{-4} was calculated. By multiplying this mobility by the factor 14, the P.D. between the particles and the medium was obtained in millivolts (for a temperature of 24°C.). The details for this calculation may be gathered from Burton[5] and Ellis.[6] The strength of the electric field in our experiments was 4.5 volts per centimeter.

II.

Experiments on the Cataphoretic P.D. *of Collodion Particles.*

The collodion particles were prepared in the following way. Merck's solution of non-flexible collodion in alcohol and ether was

[4] Northrop, J. H., *J. Gen. Physiol.*, 1921–22, iv, 629.

[5] Burton, E. F., The physical properties of colloidal solutions, 2nd edition, London, New York, Bombay, Calcutta, and Madras, 1921.

[6] Ellis, R., *Z. physik. Chem.*, 1911–12, lxxviii, 321; 1912, lxxx, 597.

poured into H_2O and stirred with a glass rod. A spongy mass of solid collodion accumulated around the rod. The solid collodion was washed a few times with H_2O and dried with filter paper. A strong solution of this collodion was then made up in chemically pure acetone (about 10 gm. in 100 cc.) and enough H_2O was added until a light cloudiness was formed. The acetone was then removed from the solution by distillation under reduced pressure, and the remaining milky fluid was centrifuged. The sediment, when stirred up with H_2O, gives a concentrated suspension of collodion particles. The suspension was centrifuged and the larger particles in the sediment were used. These particles were very convenient for the investigation of the migration as well as of the stability of the suspension.

In the following experiments 2 drops of the concentrated suspension of collodion particles were added to 50 cc. of the various solutions used, and this dilute suspension served as material for the mobility measurements. The collodion particles moved towards the anode, thus indicating that they were negatively charged. Only in the presence of salts with trivalent cations, like $LaCl_3$, was the sign of charge reversed.

In these experiments it was found that the P.D. between the collodion particles and water was a minimum when the water contained no salts and was as near as possible the point of neutrality. When acid, alkali, or a salt was added the P.D. rose rapidly with increasing concentration until a maximum was reached. This maximum depended on the nature of the electrolyte added. It was high when the anion was plurivalent and the cation univalent, and low when the cation was plurivalent and the anion monovalent. The influence of acid, alkali, and salts with monovalent ions; *e.g.*, HCl, NaOH, and NaCl, was not very different. Upon the addition of increasing concentrations of electrolytes the P.D. rose, reaching a maximum, and then dropped again upon a further rise of concentration. We will illustrate this in more detail.

The distilled water used in these experiments had a pH of about 5.8. At this pH the cataphoretic P.D. between collodion particles and water was about 22 to 30 millivolts. It was difficult to obtain more definite figures probably on account of the CO_2 error. When the distilled water was replaced by acid or alkali of different concen-

trations, the P.D. rose at first with increasing concentrations of the acid or alkali until a maximum was reached at a concentration between M/1,000 and M/500. This is shown in Fig. 1, where the ordinates

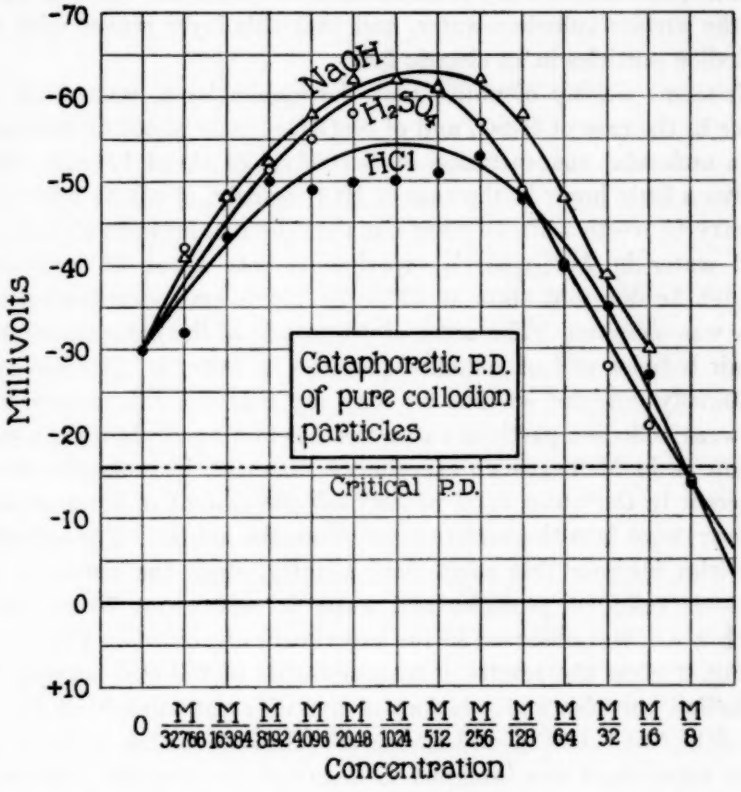

FIG. 1. Influence of acid and alkali on the cataphoretic P.D. between collodion particles and water. The original pH of water was about 5.0. The abscissæ are the concentrations of acids or alkali, the ordinates are the P.D. in millivolts. The collodion particles were negatively charged. The line marked "Critical P.D." (at 16 millivolts) is in this and the following figures the P.D. below which the collodion suspension is no longer stable.

are the P.D. and where the abscissæ are the molar concentrations of the electrolytes. The P.D. was at the maximum about 55 to 65 millivolts. With a further rise in concentrations of acid or alkali the P.D. dropped rapidly and the drop was about the same for the

acid and alkali (Fig. 1). The rise in P.D. must be due to the fact that in the case of low concentrations of NaOH an excess of OH ions and in the case of low concentrations of HCl and H_2SO_4 an excess of Cl and SO_4 ions respectively is forced into the outermost layer of water at the surface collodion-water, and that this layer moves with the collodion particles in an electric field.

It seems worthy of notice that the maximal P.D. was about the same in the case of H_2SO_4 and of NaOH, namely about 63 millivolts at a molecular concentration of electrolyte of about 1/1,024, while it was a little lower in the case of HCl; namely, about 55 millivolts. It may be worth while to point out that the P.D. between air bubbles and water in McTaggart's experiments was about 55 millivolts; though he does not state at which hydrogen ion concentration this P.D. was observed. The order of magnitude of the cataphoretic P.D. of air bubbles and of collodion particles in water is, therefore, not materially different so that we may say that the cataphoretic P.D. between collodion particles and water has in all probability the same origin as in McTaggart's experiments; namely, in molecular forces inherent in the water itself which push the cations of an electrolyte deeper down into the surface layer, than the anions. The collodion particles increase this effect only slightly, since the maximal P.D. between collodion particles and water is only about 70 millivolts. Such a P.D. was observed in the experiments recorded in Fig. 2.

Fig. 2 gives the results of measurements of the P.D. between the collodion particles and solutions of five different salts, $Na_4Fe(CN)_6$, Na_2SO_4, NaCl, $CaCl_2$, and $LaCl_3$, all solutions having a pH of 5.8. This experiment was intended to illustrate the relative influence of the valency of the anions and cations on the cataphoretic P.D. The P.D. rose upon the addition of salts with univalent cation (Na) to a maximal value of about 70 millivolts, but the concentration required to reach this maximum was least for $Na_4Fe(CN)_6$, a little higher for Na_2SO_4, and still a little higher for NaCl. A further increase of the concentration of the salts depressed the P.D., the curves dropping rapidly (Fig. 2). The maximum was but slightly higher in the case of $Na_4Fe(CN)_6$ than in the case of Na_2SO_4, and but slightly higher in the case of Na_2SO_4 than in the case of NaCl.

Fig. 2 shows also that the initial rise in the P.D. did not occur at all or occurred at a very low concentration when the salt added was $LaCl_3$, and that the rise was small when the salt added was $CaCl_2$;

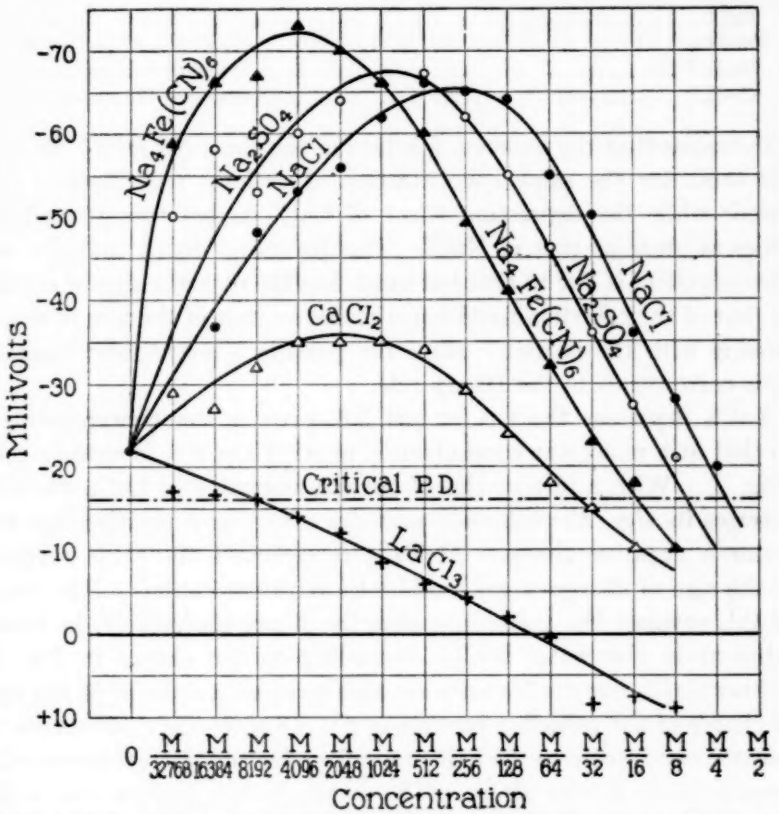

FIG. 2. Influence of $Na_4Fe(CN)_6$, Na_2SO_4, NaCl, $CaCl_2$, and $LaCl_3$ on the P.D. at pH 5.8. Addition of little salt with monovalent cation raises the P.D. to about 70 millivolts and the more rapidly the higher the valency of the anion. With $CaCl_2$ only a slight rise and with $LaCl_3$ no rise occurs in the concentrations used. In concentrations above M/64 $LaCl_3$ causes a reversal of the sign of charge of the particles.

the maximal P.D. in the latter case was 36 millivolts at a concentration of M/2,048 of the salt. After the maximum was reached the curves dropped rapidly and this drop was apparently due to the cation only. A comparison of the descending branches of the curves shows that

to bring the P.D. down from the maximum (about 70 millivolts) to *e.g.* 27.5 millivolts, the following molecular concentrations of the salts were required.

$NaCl$	M/8
Na_2SO_4	M/16
$Na_4Fe(CN)_6$	slightly less than M/32
$CaCl_2$	between M/128 and M/256

This means that the depressing action of the three Na salts is almost the same for the same concentration of cations, regardless of the anion; while the depressing effect of $CaCl_2$ is between 16 and 32 times as great as that of NaCl. This leaves no doubt that the depressing effect is due to that ion which has the opposite sign of charge to that of the collodion particle (or rather to that of the film of water moving with the particle), since the particle is negatively charged. This corresponds to the Hardy rule.

$LaCl_3$ depresses the P.D. at pH 5.8, even at low concentrations, so that at a molecular concentration of M/64 the P.D. is already zero (Fig. 2). With a further rise of the concentration of $LaCl_3$ the P.D. reverses its sign; the collodion particles assuming a positive and the water a negative charge. McTaggart observed the same reversal of the sign of charge of gas bubbles by trivalent cations. The cause of this reversal lies therefore primarily, if not exclusively, in forces inherent in the water itself. According to the curves in Fig. 2, neither $CaCl_2$ nor the Na salts are able to cause a reversal of the sign of charge of the collodion particle at pH 5.8 since the curves seem to become asymptotic to the axis of abscissæ for concentrations varying from M/16 to M/2 or above. The curves in Fig. 1 show that acids do not bring about a reversal in the concentrations used in the experiment.

The question arose whether other properties of the ion except its valency contribute to the depressing effect. The writer expected a difference in the depressing effects of LiCl, NaCl, and KCl. As a matter of fact, no difference in the influence of the three salts on the cataphoretic P.D. was noticed, as Fig. 3 shows. If differences existed they were within the limit of error in these experiments. These experiments were made at a pH of 4.7. Fig. 4 shows that the influence

of salts on the cataphoretic P.D. of collodion particles is about the same at a pH of 4.7 (Fig. 4) as at a pH of 5.8 (Fig. 2).

When the experiments were made at a higher alkalinity, namely, in a N/1,000 KOH solution (Fig. 5), the P.D. was already about 60 millivolts when no salts were added and the addition of salt could only

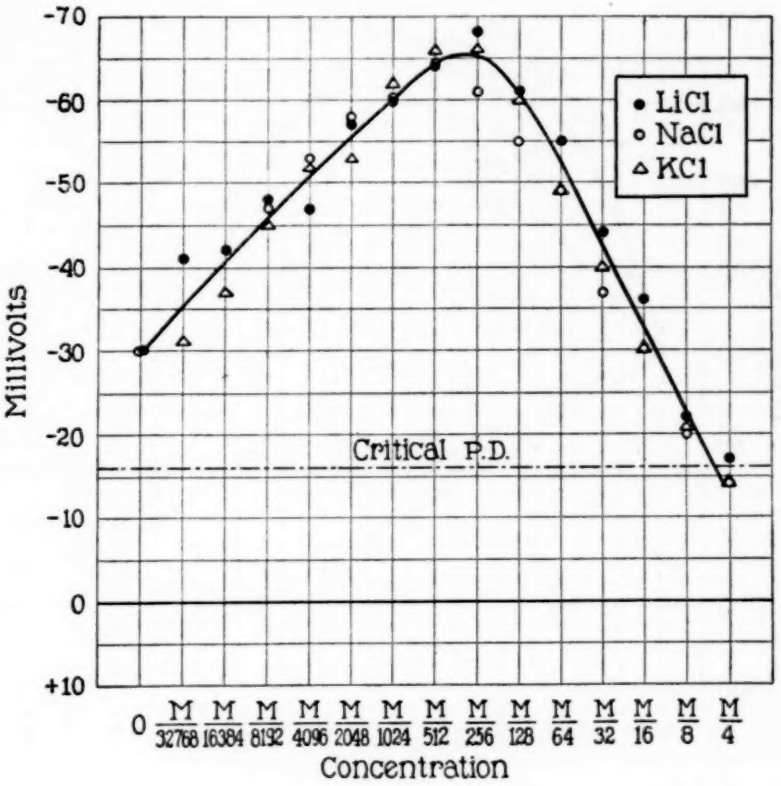

FIG. 3. Influence of LiCl, NaCl, and KCl on the P.D. at pH 4.7.

raise the P.D. to its usual maximum of about 70 millivolts. This slight rise occurred in Na_2SO_4 and $Na_4Fe(CN)_6$, but to a less amount in NaCl. In concentrations of M/256 and less, all the salts had only a depressing effect.

To bring the P.D. down to 35 millivolts in N/1,000 KOH, an approximate concentration of M/16 NaCl, of M/32 Na_2SO_4, of a little less

than M/64 $Na_4Fe(CN)_6$, and of approximately M/1,500 $CaCl_2$ was required. The depressing ion is therefore again the cation as was to be expected, since the collodion particle or rather the water film moving with it is negatively charged.

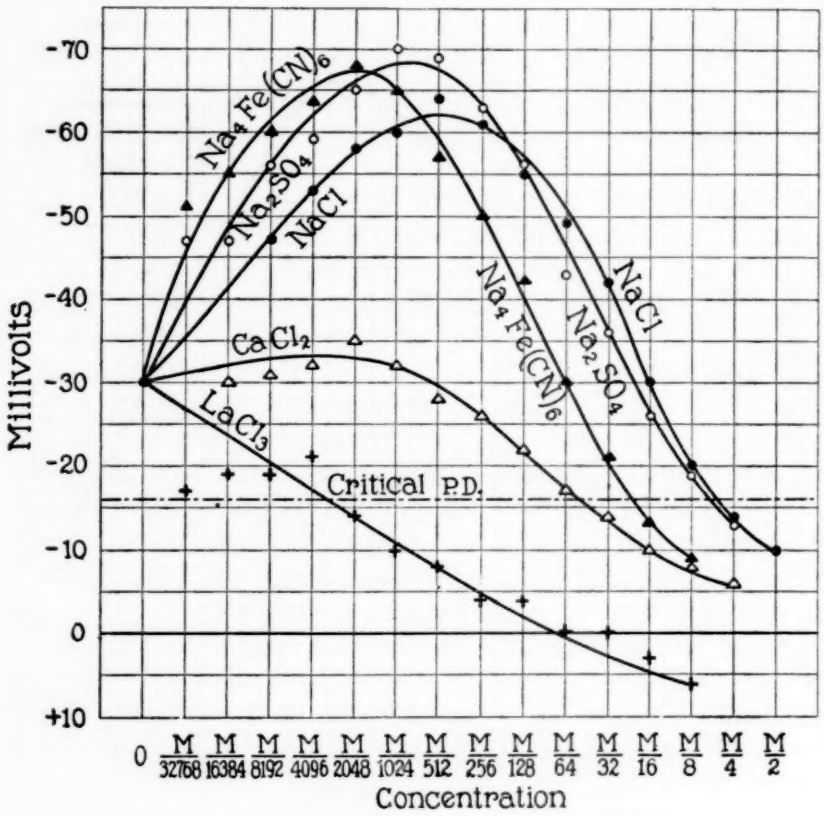

FIG. 4. Similar to Fig. 2 except that the pH was 4.7.

When the experiments were made at higher hydrogen ion concentrations, *e.g.* in N/1,000 HCl (Fig. 6), the particles had nearly their maximal charge without the addition of salt, since the P.D. was about 64 millivolts. Hence the addition of NaCl has no augmenting effect while Na_2SO_4 has a slight augmenting effect to 72 millivolts. At concentrations below M/256 the salts depress the charge of the par-

ticles. Since the particles are always negatively charged the depressing effect increases markedly with the increasing valency of the cation, as was to be expected. To depress the charge to 27.5 millivolts, a

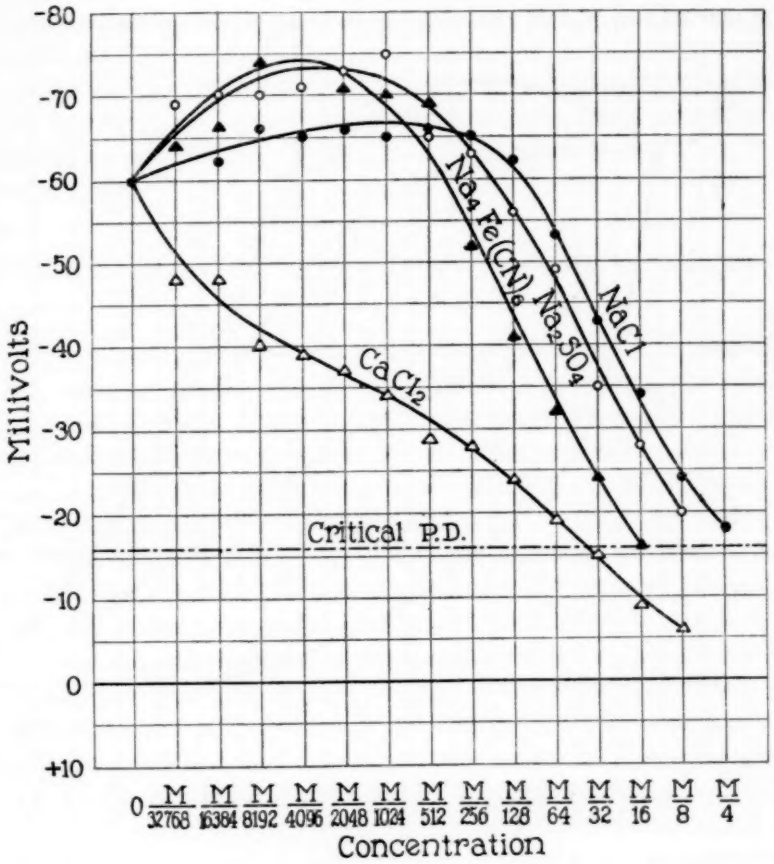

FIG. 5. Influence of salts on the cataphoretic P.D. at pH 11.0. Without salt the P.D. was already near the maximum and hence the addition of salt had only a slight augmenting effect on the P.D.

concentration of M/16 NaCl, M/256 $CaCl_2$, and M/16,384 $LaCl_3$ is required. In this acid solution $LaCl_3$ diminished the P.D. but did not bring about a reversal of the charge; perhaps for the reason that the original P.D. due to the acid was too high at the beginning.

It is often stated that H and OH ions have a greater effect on the P.D. than other ions. This was not the case in our experiments. A comparison of Fig. 1 and Fig. 2 shows that HCl, H_2SO_4, and NaOH act very much like NaCl or Na_2SO_4 on the P.D. The reason that in N/1,000 HCl or NaOH the addition of a salt no longer raises the P.D.

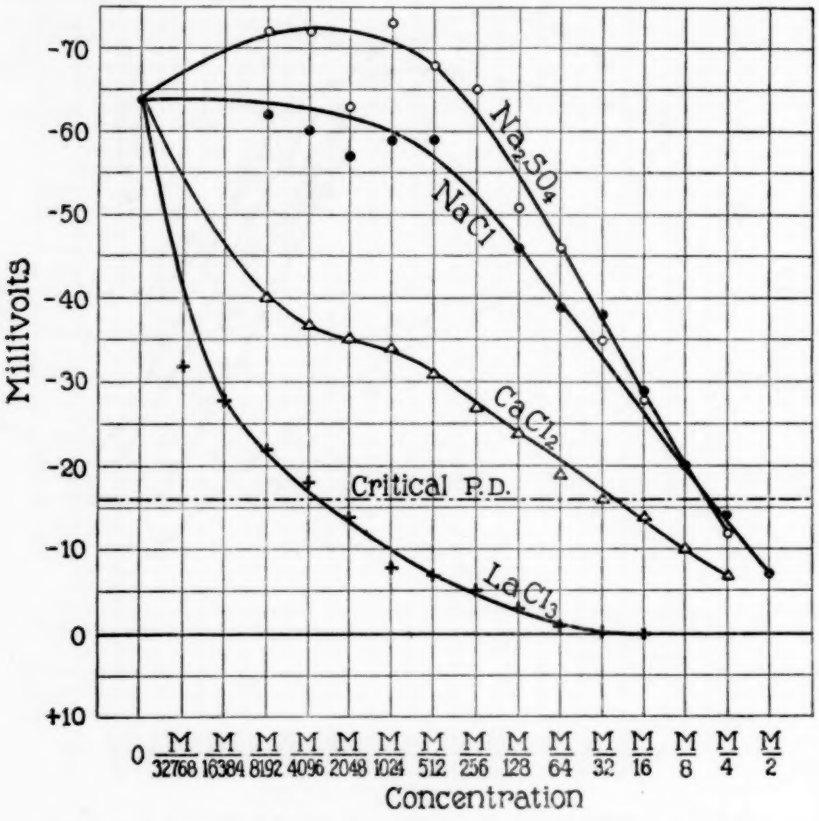

FIG. 6. Influence of salts on the P.D. at pH 3.0. (See legend of Fig. 5.)

is because the P.D. is already near the maximum before the salt is added, so that only the depressing effect of the salt becomes noticeable.

The idea that H and OH ions act more strongly than other monovalent ions is probably based on experiments with amphoteric electrolytes (where acids and alkalies bring about differences in ionization), but is apparently not true in the case of chemically inert sub-

stances like collodion. The statement that H ions act like Na ions on the cataphoretic P.D. of collodion particles is also borne out by the fact that acids do not depress the P.D. more than do Na ions, as a comparison of Figs. 1 and 2 shows. A reversal of the P.D. was observed when $LaCl_3$ was added but not when HCl or NaCl were added to the solution.

All these experiments prove, however, that it is necessary to measure the hydrogen ion concentration of the solution since otherwise the results are not strictly reproducible and comparable.

III.

The Critical P.D. *for the Precipitation of Collodion Particles from Aqueous Solutions.*

The question arose whether this cataphoretic P.D. or some other P.D. was responsible for the stability of suspensions of collodion particles in water. It can be shown that the stability of a suspension of collodion particles free from protein depends on the cataphoretic P.D., since precipitation always occurs below the same critical cataphoretic P.D. of about 16 millivolts.

When the stock suspension of collodion particles was shaken up to produce an equal distribution of particles and 1 drop of this suspension was added to 10 cc. of distilled water (of pH 5.8), the new suspension was milky when shaken up and remained so for several days. During this time the larger particles all settled and only a cloudy gray suspension was left, which gradually, after the still further settling of larger particles, gave way to a bluish opalescent suspension which lasted for many weeks ("permanently"). In this case the settling was a slow process. When 1 drop of the stock suspension was put into 10 cc. of an aqueous salt solution (also of pH 5.8) it was noticed that there existed a critical concentration of the salt, varying according to the nature of the salt, below which the suspension behaved as it did in distilled water, while in the next higher concentration a rapid, complete settling of the whole mass of collodion occurred in 12 hours or less, leaving not an opalescent but an entirely clear aqueous solution.

It was therefore comparatively easy to determine at which concentration the slow settling was replaced by a rapid settling caused by

coalescence of the small particles into larger ones. It was found that at the concentrations of salts where the rapid settling occurred, the cataphoretic P.D. between particles and aqueous solution fell below the value of about 16 millivolts; regardless of the nature of the electrolyte used for precipitation. When the P.D. was above this value, the suspension was as stable as if no electrolyte had been added; and the stability was no greater at a P.D. of 60, or 70 millivolts, than at a P.D. of 50, or 25 millivolts.

The existence of such a critical P.D. for the precipitation of suspensions agrees with the observations of Powis[7] as well as of Northrop and De Kruif.[8] Powis has made it probable that the stability of oil emulsions in aqueous solutions is destroyed when the cataphoretic P.D. between oil droplets and water is depressed below the critical value of about 30 millivolts. Northrop and De Kruif have shown that certain bacteria agglutinate when their cataphoretic P.D. is depressed by electrolytes below a critical value of 15 millivolts.

Table I gives the results of experiments on the precipitation of suspended particles of collodion by electrolytes. In one series of experiments the pH was 5.8, in a second 11.0, and in a third 3.0. In the second column of Table I are given the minimal concentrations at which precipitation was observed, *i.e.*, in which the solution became completely clear in less than 18 hours, at about 20°C., while in the fourth column are found the maximal concentrations at which the suspensions remained "permanently" stable, *i.e.* opaque for days and opalescent for weeks; in other words, where the salt caused no coalescence of particles. No attempt was made to locate the critical concentration more accurately than within the limits of concentrations given in the table, since it would probably not have been of any use in an attempt to define more sharply the real quantity of importance; namely, the critical P.D. between particles and solution. In Column 3 are found the P.D. between particles and solution at the minimal concentrations where precipitation occurred, and in the fifth column are found the P.D. of the maximal concentrations where the suspension remained stable.

[7] Powis, F., *Z. physik. Chem.*, 1914–15, lxxxix, 186.

[8] Northrop, J. H., and De Kruif, P. H., *J. Gen. Physiol.*, 1921–22, iv, 639.

It may be pointed out that the precipitating action of acids like HCl or H_2SO_4 is of the same order of magnitude as that of Na salts but not of the order of magnitude of La salts. This agrees with the

TABLE I.

Cataphoretic Charge and Stability of Suspensions of Particles of Collodion.

1.	2. Minimum concentration required for precipitation.	3. P.D. in millivolts.	4. Maximal concentration at which suspension remains stable.	5. P.D. in millivolts.
		pH 5.8		
LiCl	M/2	(10)	M/4	17
NaCl	M/2	10	M/4	14
KCl	M/4	14	M/8	21
Na_2SO_4	M/4	13	M/8	19
$Na_4Fe(CN)_6$	M/16	13	M/32	21
$MgCl_2$	M/16	11	M/32	15
$MgSO_4$	M/16	15	M/32	19
$CaCl_2$	M/32	14	M/64	17
$LaCl_3$	M/2,048	14	M/4,096	21
		pH 11.0		
NaCl	M/2		M/4	18
Na_2SO_4	M/4		M/8	20
$Na_4Fe(CN)_6$	M/16	16	M/32	24
$CaCl_2$	M/32	15	M/64	19
		pH 3.0		
NaCl	M/2	7	M/4	14
Na_2SO_4	M/4	12	M/8	(Lost.)
$CaCl_2$	M/32	16	M/64	19
$LaCl_3$	M/2,048	14	M/4,096	18
H_2SO_4	M/4		M/8	14

statement made in an earlier part of the paper that the acids act like salts with monovalent cation (*e.g.*, NaCl) on the P.D.

The average of all the P.D. values for the minimal concentrations at which precipitation occurred was 13 millivolts, while the average of all the P.D. at the concentrations at which the suspensions remained stable was 18.5 regardless of the pH. This suggests as the probable

critical value for the P.D. where precipitation commences about 16 millivolts. The actual P.D. evaluated from the mobility by cataphoresis are probably accurate only within ± 2 millivolts of this value, which explains some of the slight deviations from this value in Table I.

These measurements confirm the conclusion reached by Powis as well as by Northrop and De Kruif that there exists a critical P.D. for the stability of suspensions, this critical P.D. being about 16 millivolts for collodion particles in aqueous solutions. When the P.D. falls below this value, the particles upon colliding are no longer repelled electrostatically but may adhere to each other and coalesce (*i.e.* agglutinate or coagulate) into larger particles which rapidly sink to the bottom of the test-tube. This coalescence of the colliding particles is due to forces of attraction between certain chemical groups of their molecules. If the P.D. is larger than 16 millivolts the particles will repel each other upon colliding with sufficient force to prevent coalescence. If this critical value is once exceeded the stability of the suspension is not increased when the charge is increased. I have noticed that there is no difference in the rate of settling of a suspension of the collodion particles when the charge varies between 20 and 70 millivolts.

Since the collodion particles are generally negatively charged, it was to be expected that only the cation of the salt should be responsible for the precipitation. This is corroborated by the fact that the precipitating efficiency of salts increases rapidly with the valency of the cation. Thus for NaCl, $CaCl_2$, and $LaCl_3$ the precipitating efficiency measured by the reciprocal value of the minimal concentration required for precipitation (Column 2, Table I) is as 1:16:1,024. This valency effect is considerably greater than it would be if the P.D. responsible for the stability were due to the Donnan effect.

The question has often been raised whether that ion of a salt which has the same sign of charge as the colloidal particle will not counteract the precipitating action of the other ion. The molecular precipitating concentrations for NaCl, Na_2SO_4, and $Na_4Fe(CN)_6$ are M/2, M/4, and about M/16 respectively. In M/2 NaCl and M/4 Na_2SO_4 the concentration of cations is practically identical. If the anion had an inhibiting effect on precipitation, the concentration of Na_2SO_4

required for precipitation should be higher than M/4 which is not the case. The precipitating concentration of $Na_4Fe(CN)_6$ is even lower than that to be expected if only the Na ion acted.

It follows from this that the force preventing coalescence of the colloidal particles into larger aggregates which settle rapidly is the *cataphoretic* and no other potential difference between the particles and the aqueous solution, and that there is a critical value for this P.D. which in the case of collodion particles is about 16 millivolts. As soon as the cataphoretic P.D. falls below this value the collodion particles will coalesce. The depressing and precipitating action of a salt on the negatively charged collodion particles depends practically exclusively on the cation of the salt and increases rapidly with the increase in the valency of the cation. There exists no peptization effect of plurivalent anions.

SUMMARY AND CONCLUSIONS.

1. When collodion particles suspended in water move in an electric field they are, as a rule, negatively charged. The maximal cataphoretic P.D. between collodion particles and water is about 70 millivolts. This is only slightly more than the cataphoretic P.D. found by McTaggart to exist between gas bubbles and water (55 millivolts). Since in the latter case the P.D. is entirely due to forces inherent in the water itself, resulting possibly in an excess of OH ions in the layer of water in contact and moving with the gas bubble, it is assumed that the negative charge of the collodion particles is also chiefly due to the same cause; the collodion particles being apparently only responsible for the slight difference in maximal P.D. of water-gas and water-collodion surfaces.

2. The cataphoretic charge of collodion particles seems to be a minimum in pure water, increasing as a rule with the addition of electrolytes, especially if the cation of the electrolyte is monovalent, until a maximal P.D. is reached. A further increase in the concentration of the electrolyte depresses the P.D. again. There is little difference in the action of HCl, NaOH, and NaCl or LiCl or KCl.

3. The increase in P.D. between collodion particles and water upon the addition of electrolyte is the more rapid the higher the valency

of the anion. This suggests that this increase of negative charge of the collodion particle is due to the anions of the electrolyte gathering in excess in the layer of water nearest to the collodion particles, while the adjoining aqueous layer has an excess of cations.

4. In the case of chlorides and at a pH of about 5.0 the maximal P.D. between collodion particles and water is about 70 millivolts, when the cation of the electrolyte present is monovalent (H, Li, Na, K); when the cation of the electrolyte is bivalent (Mg, Ca), the maximal P.D. is about 35 to 40 millivolts; and when the cation is trivalent (La) the maximal P.D. is lower, probably little more than 20 millivolts.

5. A reversal in the sign of charge of the collodion particles could be brought about by $LaCl_3$ but not by acid.

6. These results on the influence of electrolytes on the cataphoretic P.D. between collodion particles and water are also of significance for the theory of electrical endosmose and anomalous osmosis through collodion membranes; since the cataphoretic P.D. is probably identical with the P.D. between water and collodion inside the pores of a collodion membrane through which the water diffuses.

7. The cataphoretic P.D. between collodion particles and water determines the stability of suspensions of collodion particles in water, since rapid precipitation occurs when this P.D. falls below a critical value of about 16 millivolts, regardless of the nature of the electrolyte by which the P.D. is depressed. No peptization effect of plurivalent anions was noticed.

The mobility measurements required for the determination of the P.D. were made by Mr. M. Kunitz.